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TITLE: Continuation of a Postdoctoral Research Associateship Program with USAMRMC

PRINCIPAL INVESTIGATOR: Judith K. Nyquist, Ph.D.

CONTRACTING ORGANIZATION: National Academy of Sciences
Washington, DC 20418

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
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FOREWORD

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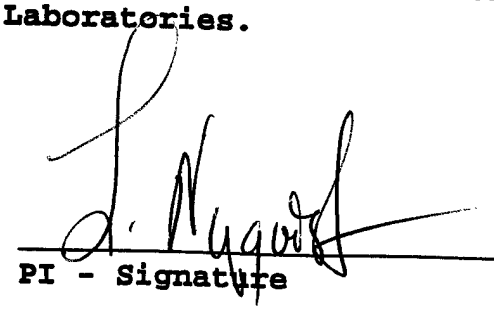

PI - Signature Date

NATIONAL RESEARCH COUNCIL

Resident Research Associateship Program

with the

U.S. Army Medical Research and Materiel Command

Status Report

October 1, 1997 to September 30, 1998

DAMD17-92-V-2002
DAMD17-95-2-5012

PUBLICITY

The NRC Research Associateship Programs for the reporting period were announced to the scientific community in the Fall of the preceding year, 1996. Publicity materials describing the NRC-AMRMC Program were distributed in November to presidents, graduate deans, and heads of appropriate science and engineering departments of all academic degree-granting institutions in the United States. These materials were also sent to Program Representatives and Associateship Advisers at the participating laboratories and to other interested persons.

REQUESTS

Application materials were distributed in response to specific requests for information about the NRC-AMRMC Associateship Program or as a result of general requests by persons whose fields of specialization appeared to be appropriate for the research opportunities available in the AMRMC laboratories.

COMPETITION

Panel reviews of applicants for the Associateship Programs, including those with the U.S. Army Medical Research and Materiel Command, are conducted in February, June, and October of each year. The following is a breakdown of the action taken with the applications during the period of the report.

	Oct 97	Feb 98	Jun 98	Total
Total Applications	17	15	19	51
Actions taken upon the above applications:				
Withdrew before Review	-	-	2	2
Deferred to Next Review	-	2	-	2
Ineligible	-	-	-	-
Incomplete Documentation	-	1	-	1
Not Approve/Not Reviewed	2	2	-1	4
Laboratory Rejection	2	-	-	2
Number of Applications Reviewed	13	10	17	40
Actions taken upon the reviewed applications:				
Non-Recommended	5	5	4	14
Recommended	8	5	13	26
Actions taken upon Recommended Applications:				
Accepted Award	6	4	10	20
Recommended/No Action	-	1	2	3
Recommended/No Funding	-	-	1	1
Alternate	-	-	-	-
Alternate with Final Turndown	1	-	-	1
Withdrew after Review/Recommended	-	-	-	-
Declined award	1	-	-	1

ASSOCIATES' ACTIVITIES

Associates who ended tenure during the report period were on tenure for an average of 28 months, with a high of 42 months and a low of 3 months. In their termination reports, the associates indicated their amount of scholarly activity while on tenure as an associate. Associates reported the following activities:

69	Domestic Presentations	16	International Presentations
38	Published Articles in Refereed Journals	5	Patents applied for

After completing their tenure associates, indicated their future plans as follows:

8	Research - National government	-	Non-Profit
1	Administration, Fed, State or Local	-	Self-employed
2	Remain at Host Agency	1	Unemployed
-	Different NRC Sponsoring Agency	1	Industry
1	College or University Professor	-	Another Post-Doctorate
-	Student	6	Other/No Information Provided

Associates on tenure as of October 1, 1997 are citizens of the following countries:

England, U.K.	1	Nigeria	2
Ethiopia	1	People's Republic of China	6
India	6	Peru	1
Israel	2	Russia	1
Latvia	1	United States	23

Other information about the associates activities can be found in the following attachments and appendix.

Attachment 1 is a list of Associates who terminated their appointments during the period of October 1, 1997 through September 30, 1998. It includes the Associates' labs, their starting and termination dates, and the names of their Advisers. Associates are required to submit reports upon termination (attached to this report), and Advisers are asked to submit a final evaluation of each Associate. Associates who have not submitted a termination report have received a follow-up letter.

Attachment 2 provides a roster of Associates on Tenure as of October 1, 1998. This listing includes the Associate's Adviser, Division, start and expected termination date, and country of citizenship.

Attachment 3 lists the applicants who received awards during the period of October 1, 1997 through September 30, 1998. It includes the title of their Research Proposals.

Attachment 4 provides a roster of all recommended candidates by category (i.e. accepted, no funding, etc.). This report includes information about the recommended candidate's education, proposed research, starting date and adviser.

Attachment 5 details a cross tabulation of how many Associates were on tenure for the year in by center for each quarter within the report period and other yearly periods.

The *Appendix* contains the copies of the "Termination Reports" received from terminating associates.

Associates Who Ended Tenure 10/1/97 - 9/30/98 Attachment 1

U.S. Army Medical Research and Materiel Command

Associate Name + Adviser		Center	Tenure Dates Start	End	Termination Report	Adviser Report
Abugo, Omoefe Oghenera. Dr. Victor W Macdonald	(S)	Walter Reed Army Institute of Research	4/06/95	10/05/97	Not Recd	Not Recd
Asermely, Karen E. Dr. Michael Adler		Medical Res Inst of Chemical Defense	5/22/95	11/07/97	Received	Received
Behonick, George Stanley Dr. Steven I Baskin		Medical Res Inst of Chemical Defense	8/04/97	9/30/98	Not Recd	Not Recd
Bhattacharjee, Apurba K Dr. Jean M Karle	(S)	Walter Reed Army Institute of Research	7/10/95	7/09/98	Received	Received
Crise, Bruce Jeffrey Dr. Michael D Parker		Medical Research Institute for Infectious Diseases	11/15/96	10/28/97	Received	Not Recd
Das, Rina Dr. Marti Jett	(S)	Walter Reed Army Institute of Research	10/01/96	9/30/98	Received	Received
Ding, Xuan Zhou Dr. Juliann G Kiang	(S)	Walter Reed Army Institute of Research	10/03/94	1/02/98	Not Recd	Received
Eze, Michael Okechukwu Dr. David L Hoover	(S)	Walter Reed Army Institute of Research	10/03/94	10/02/97	Received	Not Recd
Fried, Michal Dr. Patrick E Duffy		Walter Reed Army Institute of Research	1/11/95	7/10/98	Received	Received
Gouvea, Vera S. Dr. Bruce L Innis	(S)	Walter Reed Army Institute of Research	10/03/94	2/28/98	Received	Received
Higgins, James A. Dr. M S Ibrahim		Medical Research Institute for Infectious Diseases	1/15/97	12/15/97	Received	Received
Hooper, Jay William Dr. Connie S Schmaljohn		Medical Research Institute for Infectious Diseases	7/05/95	12/31/97	Received	Received
Lin, Yu Dr. Joseph B Long		Walter Reed Army Institute of Research	7/18/94	1/17/98	Received	Not Recd
Meyer, Barbara J Dr. Connie S Schmaljohn		Medical Research Institute for Infectious Diseases	2/01/95	1/31/98	Received	Received
Morris, Jim Dr. Tsung-Ming A Shih		Medical Res Inst of Chemical Defense	9/11/95	9/10/98	Received	Not Recd
Pierson, Vicki Lynn D Dr. Patricia L Worsham		Medical Research Institute for Infectious Diseases	9/05/95	1/16/98	Received	Not Recd
Pushko, Peter Dr. Jonathan F Smith		Medical Research Institute for Infectious Diseases	5/20/94	11/19/97	Received	Not Recd
Reddy, Shanker P Dr. Susan L Welkos	(S)	Medical Research Institute for Infectious Diseases	3/03/97	3/02/98	Received	Received
Ryu, Hyoik Dr. Frederick J Cassels	(S)	Walter Reed Army Institute of Research	10/03/94	6/30/98	Not Recd	Not Recd
Saikh, Kamal Uddin Dr. Robert G Ulrich	(S)	Medical Research Institute for Infectious Diseases	4/03/95	4/02/98	Received	Received
Santhanam, Kausalya Dr. Jayasree Nath		Walter Reed Army Institute of Research	1/05/98	7/03/98	Received	Received
Shitzer, Avraham Dr. Richard R Gonzalez	(S)	U.S. Army Research Institute of Environmental Medicine	8/12/96	9/25/98	Received	Not Recd
Sina, Barbara Jean Dr. Kenneth J Linthicum	(S)	Research Institute of Medical Sciences	6/03/98	9/02/98	Not Recd	Not Recd

+ (S) indicates the associate was a Senior.

Associates Who Ended Tenure 10/1/97 - 9/30/98 Attachment 1

U.S. Army Medical Research and Materiel Command

Associate Name + Adviser	Center	Tenure Dates Start	End	Termination Report	Adviser Report
Wasieloski, Leonard P Dr. Kevin Anderson	Medical Research Institute for Infectious Diseases	4/24/97	4/14/98	Received	Received
Yeghiayan, Karine Sylva Dr. Harris R Lieberman	U.S. Army Research Institute of Environmental Medicine	9/08/97	1/09/98	Received	Not Recd

25 Associates Listed

+ (S) indicates the associate was a Senior.

Associates On Tenure October 1, 1998 **Attachment 2**

U.S. Army Medical Research and Materiel Command

Name + Adviser	Center Citizenship	Starting Date	Ending Date
Ahmed, Syed Ashraf (S) Dr. Leonard A Smith	Medical Research Institute for Infectious Diseases United States	8/18/97	8/17/99
Baranyi, Lajos (S) Dr. C. R Alving	Walter Reed Army Institute of Research Hungary	6/22/98	6/21/99
Byrd, Wyatt (S) Dr. Frederick J Cassels	Walter Reed Army Institute of Research United States	1/20/98	1/19/99
Cerasoli, Douglas Mark Dr. David E Lenz	Medical Res Inst of Chemical Defense United States	7/20/98	7/19/99
Chakrabarti, Arun Kumar (S) Dr. Prabhati Ray	Walter Reed Army Institute of Research US Permenant Resident	5/30/96	12/14/98
Chen, Shin-Lin Dr. John W Huggins	Medical Research Institute for Infectious Diseases United States	10/01/97	10/16/98
Cui, Ping Dr. Frank C Tortella	Walter Reed Army Institute of Research People'S Republic Of China	7/28/97	7/27/99
Dailey, Frank (S) Dr. Arthur M Friedlander	Medical Research Institute for Infectious Diseases United States	11/13/96	11/12/99
Dutta, Sheetij Dr. David E Lanar	Walter Reed Army Institute of Research India	7/27/98	7/26/99
Erwin, James Lawrence Dr. Tran C Chanh	Medical Research Institute for Infectious Diseases United States	8/10/98	8/09/99
Feaster, Shawn Ray Dr. Bhupendra P Doctor	Walter Reed Army Institute of Research United States	2/03/97	2/02/99
Fegeding, Konstantin V. Dr. Jeenan Tseng	Walter Reed Army Institute of Research Russia	10/16/95	1/15/99
Fernandez-Prada, Carmen Maria Dr. David L Hoover	Walter Reed Army Institute of Research Peru	3/14/97	3/13/99
Guebre Xabier, Mimi (S) Dr. Urszula Krzych	Walter Reed Army Institute of Research US Permenant Resident	5/20/96	5/19/99
Guttieri, Mary Charity Dr. Connie S Schmaljohn	Medical Research Institute for Infectious Diseases United States	10/06/95	11/05/98
Hatfill, Steven Jay (S) Dr. John W Huggins	Medical Research Institute for Infectious Diseases United States	9/18/97	9/17/99
Hensley, Lisa Ellen Dr. Peter B Jahrling	Medical Research Institute for Infectious Diseases United States	9/01/98	8/31/99
Jenner, Jennifer Louise Dr. Harris R Lieberman	U.S. Army Research Institute of Environmental M United States	6/01/98	5/31/99
Jensen, Melody Janet (S) Dr. Leonard A Smith	Medical Research Institute for Infectious Diseases United States	8/10/98	8/09/99
Kamrud, Kurt Iver Dr. Connie S Schmaljohn	Medical Research Institute for Infectious Diseases United States	8/05/96	8/04/99
Keller, James Erich Dr. Michael Adler	Medical Res Inst of Chemical Defense United States	7/01/96	6/30/99
Khan, Akbar S. (S) Dr. Robert G Ulrich	Medical Research Institute for Infectious Diseases United States	9/15/98	9/14/99

*Indicates that the associate started tenure between 10/1/97 and 9/30/98.
(S) Associate is a Senior.

Associates On Tenure October 1, 1998 **Attachment 2**

U.S. Army Medical Research and Materiel Command

Name + Adviser	Center Citizenship	Starting Date	Ending Date
Li, Guo Dr. Harry Zwick	Walter Reed Army Institute of Research US Permenant Resident	9/16/96	9/15/99
Lumley, Lucille Ann Dr. James L Meyerhoff	Walter Reed Army Institute of Research United States	1/03/96	1/02/99
Luo, Chunyuan Dr. Bhupendra P Doctor	Walter Reed Army Institute of Research People'S Republic Of China	3/12/96	3/11/99
Ma, Da Dr. Mustapha Debboun	Walter Reed Army Institute of Research US Permenant Resident	1/29/97	12/28/98
Moran, Daniel Sender Dr. Kent B Pandolf	U.S. Army Research Institute of Environmental M Israel	8/01/97	7/31/99
Palmer, Dupeh Rachel O Dr. Urszula Krzych	Walter Reed Army Institute of Research England, U.K.	11/27/95	5/26/99
Peel, Sheila Anne Dr. Rodger K Martin	Walter Reed Army Institute of Research United States	8/01/96	7/31/99
Phillips, James Boyce, Jr Dr. Frank C Tortella	Walter Reed Army Institute of Research United States	10/06/97	10/05/99
Price, Elvis Odin Dr. Ming L Shih	Medical Res Inst of Chemical Defense United States	1/15/98	1/14/00
Ruff, Albert Leonard Dr. Connie S Schmaljohn	Medical Research Institute for Infectious Diseases United States	3/30/98	3/29/99
Waitumbi, John Njenga (S) Dr. Jose A Stoute	Walter Reed Army Institute of Research Kenya	2/09/98	2/08/99
Weeks, Steven Douglas Dr. Susan L Welkos	Medical Research Institute for Infectious Diseases United States	5/04/98	5/03/99
Wilson, Julie Ann Dr. Mary K Hart	Medical Research Institute for Infectious Diseases United States	3/24/97	3/23/99
Xiang, Charlie Chunsheng (S) Dr. Kevin Anderson	Medical Research Institute for Infectious Diseases Canada	5/15/98	5/14/99
Yadava, Anjali Dr. Christian F Ockenhouse	Walter Reed Army Institute of Research US Permenant Resident	1/02/96	1/01/99

*Indicates that the associate started tenure between 10/1/97 and 9/30/98.
(S) Associate is a Senior.

Name/
Research Title

October 1997 Awardees

Awardees Listed 6

Baranyi, Lajos
Improving Liposome Enclosed Hemoglobin Based Blood Substitutes

Byrd, Wyatt
Oral, Adjuvanted Trivalent Vaccine Directed Against the Enteric Pathogens, Entertoxigenic E. coli, Camphylobacter and Shigella

Price, Elvis O
Investigations of Sulfur Mustard Adducts to Proteins via On-Line Capillary Zone Electrophoresis/Electrospray Ionization Mass Spectrometry

Ruff, Albert L
Enhancing the Immune Response to Ebola Antigens

Sina, Barbara J
Preparation of Reagents for the Isolation of P. Vivax Non-CSP Antigen Genes

Waitumbi, John N
Identification of Immune-Mediated Mechanisms Responsible for Destruction of Uninfected Red Blood Cells in Plasmodium Falciparum Malaria

February 1998 Awardees

Awardees Listed 4

Dutta, Sheetij
Production of Recombinant Vaccine Candidate Antigens of the Human Malarial Parasite Plasmodium Vivax

Hensley, Lisa E
Study of Ebola-Reston and Simian Hemorrhagic Fever Virus Co-Infections

Jenner, Jennifer L
The Effects of Antioxidants on Eccentric Exercise-induced Muscle Injury and Performance

Weeks, Steven D
Analysis of the Mechanism of Antigen-Induced Supression of Leukocyte TNF-Alpha and Interferon-Gamma Secretion

June 1998 Awardees

Awardees Listed 10

Bosio, Christopher F
Nucleic Acid Immunization with Self-Replicating Messenger RNAs

Name/
Research Title

Cerasoli, Douglas M
Analysis of the Structure and Function of Anti-Organophosphorus Catalytic Antibodies

Darko, Christian A
Requirement for Replicating Native Structure to Induce Protective Immunity Against Malaria Parasites with Recombinant MSP-1 42

Dekonenko, Alexander E
Development of a Membrane Assay to Detect and Differentiate Known Pathogenic Hantaviruses

Erwin, James L
Immune Intervention Against the Subversion of Macrophages by Anthrax Lethal Toxin

Fonseca, Dina M
Vector Mediated Selection in Human Malaria: Partitioning of Parasite Genetic Diversity Across Species and Across Populations within Species of Medically Important Anopheles in Brazil

Jensen, Melody J
Expression of the Light Chain of Clostridium Toxin as a Soluble Form in E. coli

Khan, Akbar S
Optimizing Immune Responses to Recombinant Vaccines: Role of Activated Dendvitic Cells and Vaccine Development by Phage Display Selection

Liu, Liang M
Relationship of Nitric Oxide Induced Vascular Hyporesponsiveness with Adrenergic Alpha and Beta Receptors Following Hemorrhagic Shock

Peng, Daizhi
The Effects of NF-kB and IkB on the Imbalance Between Inflammatory and Antiinflammatory Cytokines During Hemorrhage and Trauma

Total Associates Listed for Lab 20

U.S. Army Medical Research and Materiel Command

October 1997

1- Recommended

TERRY-KOROMA, BARBARA A — Ph.D. Date: 1991
Citizenship: United States — North Carolina State U-Raleigh
Adviser: Dr. Marti Jett
Research Field: Molecular Basis Biol Phenom
Research Title: The Effect of Neonatal Environmental Estrogen Exposure on Mammary Gland Development and the Multi-Step Process Associated with Transformation for Normal to Tumorigenic in a Murine Model System

A- Accepted Award (6 Applicants listed)

BARANYI, LAJOS — Ph.D. Date: 1986
Citizenship: Hungary — Szeged, U Of
Adviser: Dr. C. R Alving — Actual Starting Date: 6/22/98
Research Field: Physiological Science — Termination Date: 6/21/99
Research Title: Improving Liposome Enclosed Hemoglobin Based Blood Substitutes

BYRD, WYATT — Ph.D. Date: 1991
Citizenship: United States — University of Georgia
Adviser: Dr. Frederick J Cassels — Actual Starting Date: 1/20/98
Research Field: Gastroenterology — Termination Date: 1/19/99
Research Title: Oral, Adjuvanted Trivalent Vaccine Directed Against the Enteric Pathogens, Entertoxigenic E. coli, Camphylobacter and Shigella

PRICE, ELVIS O — Ph.D. Date: 1996
Citizenship: United States — Howard University/DC
Adviser: Dr. Ming L Shih — Actual Starting Date: 1/15/98
Research Field: Analytical Chemistry — Termination Date: 1/14/00
Research Title: Investigations of Sulfur Mustard Adducts to Proteins via On-Line Capillary Zone Electrophoresis/Electrospray Ionization Mass Spectrometry

RUFF, ALBERT L — Ph.D. Date: 1997
Citizenship: United States — Johns Hopkins U-Medical Insts./MD
Adviser: Dr. Connie S Schmaljohn — Actual Starting Date: 3/30/98
Research Field: Immunology — Termination Date: 3/29/99
Research Title: Enhancing the Immune Response to Ebola Antigens

SINA, BARBARA J — Ph.D. Date: 1982
Citizenship: United States — University of Southern California
Adviser: Dr. Kenneth J Linthicum — Actual Starting Date: 6/03/98
Research Field: Parasitology — Termination Date: 9/02/98
Research Title: Preparation of Reagents for the Isolation of P. Vivax Non-CSP Antigen Genes

WAITUMBI, JOHN N — Ph.D. Date: 1992
Citizenship: Kenya — Nairobi, U Of
Adviser: Dr. Jose A Stoute — Actual Starting Date: 2/09/98
Research Field: Infectious Diseases — Termination Date: 2/08/99
Research Title: Identification of Immune-Mediated Mechanisms Responsible for Destruction of Uninfected Red Blood Cells in Plasmodium Falciparum Malaria

8- Declined

RAVINDRAN, ARIPPA — Ph.D. Date: 1984
Citizenship: United States — Jawaharlal Nehru Tech Univ/India
Adviser: Dr. Michael Adler
Research Field: Neurotoxicology
Research Title: Mechanism of Action of Quinolines on Botulinum Neurotoxin Channels in Planar Lipid Bilayers

Y- Alternate with Final Turndown

IVANOVA, VESSELA S — Ph.D. Date: 1989
Citizenship: Bulgaria — Bulgarian Academy of Sciences
Adviser: Dr. Ronald Rosenberg
Research Field: Entomology
Research Title: Proteins Involved in the Process of Exflagelation of the Malaria Parasite Plasmodium

February 1998

1- Recommended

HUANG, XIAOZHE — Ph.D. Date: 1982
Citizenship: People's Republic of China — Nanjing Medical College/China
Adviser: Dr. Luther E Lindler
Research Field: Microbial Genetics
Research Title: Molecular Genomic Characterization of Yersinis Pestis: a Reemerging Infectious Disease

A- Accepted Award (4 Applicants listed)

DUTTA, SHEETIJ — Ph.D. Date: 1998
Citizenship: India — Lucknow, U Of
Adviser: Dr. David E Lanar — Actual Starting Date: 7/27/98
Research Field: Parasitology — Termination Date: 7/26/99
Research Title: Production of Recombinant Vaccine Candidate Antigens of the Human Malarial Parasite Plasmodium Vivax

HENSLEY, LISA E — Ph.D. Date: 1997
Citizenship: United States — U of North Carolina-Chapel Hill
Adviser: Dr. Peter B Jahrling — Actual Starting Date: 9/01/98
Research Field: Pathobiology — Termination Date: 8/31/99
Research Title: Study of Ebola-Reston and Simian Hemorrhagic Fever Virus Co-Infections

JENNER, JENNIFER L — Ph.D. Date: 1998
Citizenship: United States — Tufts University/MA
Adviser: Dr. Harris R Lieberman — Actual Starting Date: 6/01/98
Research Field: Nutrition — Termination Date: 5/31/99
Research Title: The Effects of Antioxidants on Eccentric Exercise-induced Muscle Injury and Performance

WEEKS, STEVEN D — Ph.D. Date: 1998
Citizenship: United States — Georgetown University/DC
Adviser: Dr. Susan L Welkos — Actual Starting Date: 5/04/98
Research Field: Immunology — Termination Date: 5/03/99
Research Title: Analysis of the Mechanism of Antigen-Induced Supression of Leukocyte TNF-Alpha and Interferon-Gamma Secretion

June 1998

1- Recommended (2 Applicants listed)

LACASSE, RACHEL A — Ph.D. Date: 1998
Citizenship: United States — University of Montana
Adviser: Dr. Alan L Schmaljohn
Research Field: Virology
Research Title: Marburg Virus and Host Cell Interactions

SANDERS, MARTIN L — Ph.D. Date: 1997
Citizenship: United States — Johns Hopkins University/MD
Adviser: Dr. Peter B Jahrling
Research Field: Viral Immunology
Research Title: Characterization of Mechanisms of Pathogenesis in Three Hemorrhagic Fever Agents: Development of a Generic Model for Hemorrhagic Fever Pathology and Intervention

A- Accepted Award (10 Applicants listed)

BOSIO, CHRISTOPHER F — Ph.D. Date: 1998
Citizenship: United States — Colorado State University
Adviser: Dr. Jonathan F Smith — Expected Starting Date: 11/01/98
Research Field: Virology — Termination Date: 10/31/99
Research Title: Nucleic Acid Immunization with Self-Replicating Messenger RNAs

U.S. Army Medical Research and Materiel Command

CERASOLI, DOUGLAS M — Ph.D. Date: 1996
Citizenship: United States — University of Pennsylvania
Adviser: Dr. David E Lenz — Actual Starting Date: 7/20/98
Research Field: Molecular Immunology — Termination Date: 7/19/99
Research Title: Analysis of the Structure and Function of Anti-Organophosphorus Catalytic Antibodies

DARKO, CHRISTIAN A — Ph.D. Date: 1998
Citizenship: Ghana — University of Tokyo/Japan
Adviser: Dr. Jeffrey A Lyon — Actual Starting Date: 11/02/98
Research Field: Immunology — Termination Date: 11/01/99
Research Title: Requirement for Replicating Native Structure to Induce Protective Immunity Against Malaria Parasites with Recombinant MSP-1 42

DEKONENKO, ALEXANDER E — Ph.D. Date: 1996
Citizenship: Russia — Inst Polio & Virus Enceph/Russia
Adviser: Dr. Connie S Schmaljohn — Actual Starting Date: 11/02/98
Research Field: Molecular Virology — Termination Date: 11/01/99
Research Title: Development of a Membrane Assay to Detect and Differentiate Known Pathogenic Hantaviruses

ERWIN, JAMES L — Ph.D. Date: 1993
Citizenship: United States — State U of New York-Stony Brook
Adviser: Dr. Tran C Chanh — Actual Starting Date: 8/10/98
Research Field: Immunology — Termination Date: 8/09/99
Research Title: Immune Intervention Against the Subversion of Macrophages by Anthrax Lethal Toxin

FONSECA, DINA M — Ph.D. Date: 1996
Citizenship: Portugal — University of Pennsylvania
Adviser: Dr. Richard C Wilkerson — Actual Starting Date: 12/15/98
Research Field: Entomology — Termination Date: 12/14/99
Research Title: Vector Mediated Selection in Human Malaria: Partitioning of Parasite Genetic Diversity Across Species and Across Populations within Species of Medically Important Anopheles in Brazil

JENSEN, MELODY J — Ph.D. Date: 1988
Citizenship: United States — University of California-Davis
Adviser: Dr. Leonard A Smith — Actual Starting Date: 8/10/98
Research Field: Biotechnology — Termination Date: 8/09/99
Research Title: Expression of the Light Chain of Clostridium Toxin as a Soluble Form in E. coli

KHAN, AKBAR S — Ph.D. Date: 1989
Citizenship: United States — University of Oklahoma
Adviser: Dr. Robert G Ulrich — Actual Starting Date: 9/15/98
Research Field: Infectious Diseases — Termination Date: 9/14/99
Research Title: Optimizing Immune Responses to Recombinant Vaccines: Role of Activated Dendvitic Cells and Vaccine Development by Phage Display Selection

LIU, LIANG M — Ph.D. Date: 1994
Citizenship: People's Republic of China — Third Military Medical Col/China
Adviser: Dr. Michael A Dubick — Expected Starting Date: 3/05/99
Research Field: Traumatology — Termination Date: 3/04/00
Research Title: Relationship of Nitric Oxide Induced Vascular Hyporesponsiveness with Adrenergic Alpha and Beta Receptors Following Hemorrhagic Shock

PENG, DAIZHI — Ph.D. Date: 1994
Citizenship: People's Republic of China — Third Military Medical Col/China
Adviser: Dr. Michael A Dubick — Expected Starting Date: 12/18/98
Research Field: Traumatology — Termination Date: 12/17/99
Research Title: The Effects of NF-kB and IkB on the Imbalance Between Inflammatory and Antiinflammatory Cytokines During Hemorrhage and Trauma

Z- Recommended/No Funding

HUAN, JING-NING — Ph.D. Date: 1987
Citizenship: People's Republic of China — Shanghai Second Medical Univ
Adviser: Dr. Albert T McManus
Research Field: Surgery
Research Title: Changes of Macrophage Stats and NF-kB Activities in Burned Rats

On Tenure Report by Quarter and Center | For the year starting **October 1, 1997** | **Attachment 5**

U.S. Army Medical Research and Materiel Command

Center	Number of Associates on tenure as of					
	10/1/96	10/1/97	1/1/98	4/1/98	7/1/98	10/1/98
Medical Res Inst of Chemical Defense	4	4	3	4	4	3
Medical Research Institute for Infectious Diseases	15	16	12	10	10	14
Research Institute of Medical Sciences	1	-	-	-	1	-
U.S. Army Research Institute of Environmental Medicine	3	2	2	1	2	2
Walter Reed Army Institute of Research	32	22	22	21	21	18
	55	44	39	36	38	37

~~952190~~ 9542190

11420 Stewart Lane Apt. D-1
Silver Spring, MD 20904

January 28, 1998
page 1(4)

Judith Nyquist, Ph.D.
National Research Council
Associateship Programs
2101 Constitutional Ave, NW, TJ 2114
Washington, DC 20418

Dear Dr. Nyquist:

Enclosed is my final report regarding my tenure as a National Research Council Fellow. It has been a pleasure working with you during my postdoctoral fellowship. I hope to keep in touch with you as my future endeavors unfold and see you at professional meetings.

(2) **Karen Elizabeth Asermely, Ph.D.**

(3) **Laboratory:** United States Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, Maryland 21010-5425.

(4) **Dates of tenure:** May 22, 1995 to November 7, 1997.

(5) **Title of Research Proposal:** A Study of Metalloendopeptidase Inhibitors on Botulinum Toxin Treated Synaptic Proteins in Cell Free Systems.

(6) **Research Advisor:** Dr. Michael Adler

(7) No, I am not on leave from a professional post.

(8) **Professional Society Membership:** New member of the Interagency Botulinum Research Coordinating Committee of the US, American Society of Neurochemistry, International Society of Neurochemistry, Ordinary member of Association of Pharmacology and Experimental Therapeutics

(9) **Professional Travel During Tenure:**
a.PerSeptive BioSystems Inc. 500 Old Connecticut Path Framingham MA 01701
Awarded a Certificate of Achievement for satisfactory completion of the PepSynthesizer Workstation Training Class from September 27-29, 1995
b.Attended a Conference on Capillary Electrophoresis from October 23-25, 1995 at Hood College, Frederick, Maryland 21701
c.Presented a poster at the Conference of the Eighth International Symposium on Capillary Electrophoresis from January 21-25, 1996 in Orlando, Florida.

d.Attended the American Society of Neurochemistry 27th Annual Meeting from March 2-6, 1996 in Philadelphia, PA.
e.Presented a Poster at the U.S. Army Medical Bioscience Review, Annual Meeting from May 12-16, 1996 in Baltimore, MD.
f.Attended Society of Neuroscience in Washington DC in Oct 1996
g.Presented a poster at the Society of Neuroscience Annual Meeting in New Orleans, LA in Oct 1997.
h.Presented a slide presentation at the Annual Interagency Botulinum Toxin Research Coordinating Committee on Nov. 14, 1997 in Bethesda, MD.

(10) There were no seminars or lectures delivered at universities or institutes.

(11) Summary of Research During Tenure: A new capillary zone electrophoresis method was established (new method in literature) for a TSB-51, 51 amino acid fragment of vesicle associated membrane protein II-thioredoxin fusion protein, and for a (VAMP-51)51 amino acid fragment of vesicle associated membrane protein II. The TSB-51 protein was separated by CZE, fraction collected and sequenced by the Edman method which resulted in a publication in 1997. The TSB-51 after being validated on CZE was used in botulinum toxin experiments by others.

The VAMP51 and two cleavage products (VAMP18 & VAMP 33) were synthesized by FMOC peptide synthesis and polyclonal antibodies to VAMP51 were made and tested by ELISA for cross reaction to VAMP18 and VAMP33. Each peptide, VAMP51, VAMP18 and VAMP33 were separated on a newly developed CZE method to identify the migration times of their appearance on the epherogram. This method includes the use of lysyl-glycine as an external standard for all VAMP51 separations expressed in a calibration curve of the Ratio [peak area of VAMP51/ corrected peak area Lys-Gly] versus VAMP51 concentration.

Botulinum toxin B light chain was tested over various concentrations to optimize the cleavage of VAMP51; however, because of stability (or lack thereof) this was not reproducible. The assay of Botulinum toxin B cleavage was developed using the whole toxin, BoTX/B. This is very reproducible and a time course of cleavage was done using 20 nM BoTX/B. The time at one half cleavage was determined to be 90 min with 100 μM VAMP51 and 20 nM BoTX/B. The cleavage products obtained on the CZE (observed) migrated correctly when compared to the pure synthesized VAMP18 and VAMP33, validating the cleavage of VAMP51, in vitro on CZE.

The Km of VAMP51 was determined using BoTX/B. This was found to be 65 μM VAMP51 using 20 nM BoTX/B at 25C. A new on-line kinetic study was developed to follow the rate of cleavage over various concentrations of VAMP51 (38 uM-500 uM). Botulinum Toxin B was added to VAMP51 in appropriate buffer and at the time of mixing the sample was injected into the CZE approximately every 14 min over 2 hours. This enables one to monitor the rate of cleavage of VAMP51 by BoTX/B over time. This is the first report of the use of CZE to monitor botulinum toxin and its substrate which is involved in vesicular release of acetylcholine.

In the host laboratory, USAMRICD, the main mission was to develop the CZE method and to identify new inhibitors of BoTX/B. Two compounds were tested for inhibition of BoTX/B mediated cleavage of VAMP51. These are lisinopril, an angiotensin converting enzyme (ACE) inhibitor and substance P.

Initially the lisinopril was shown to delay the cleavage of VAMP51 by BoTX/B. These results are being confirmed followed by a dose response curve. This is the 2nd publication listed as in preparation.

Substance P inhibited the cleavage of VAMP51 by BoTX/B. A dose response curve and IC50 are being repeated and validated. Two analogues of substance P to be tested are: a mutated substance P and a fragment of substance P. These are ongoing in the laboratory presently. These results constitute the 3rd paper under publications "in preparation".

(12) Research in Progress: There will be continuing collaboration between Dr. Brennie Hackley, Dr. Michael Adler, Dr. Clarence Broomfield and Dr. Frank Cann. There are many new insights which have been found in these studies on which I will be collaborating on future papers.

(13) Presentations at Scientific Meetings/Conferences:

a.Presented a poster at the Conference of the Eighth International Symposium on Capillary Electrophoresis from January 21-25, 1996 in Orlando, Florida.

b.Presented a Poster at the U.S. Army Medical Bioscience Review, Annual Meeting from May 12-16, 1996 in Baltimore, MD.

c.Presented a poster at the Society of Neuroscience in New Orleans, LA in Oct 1997.

d.Presented a slide presentation at the 1997 Meeting of the Interagency Botulism Research Coordinating Committee from November 12-14, 1997.

(14) Publications in Peer-Reviewed Journals:

"Identification of a recombinant synaptobrevin-thioredoxin fusion protein by capillary zone electrophoresis using laser-induced fluorescence detection" Karen E. Asermely, Clarence A. Broomfield, Janet Nowakowski, Bernard C. Courtney, Michael Adler. Journal of Chromatography B695 (1997) 67-75.

"Measurement of a Synaptobrevin-Thioredoxin Fusion Protein (VAMPII(51aa)-T by Capillary Zone Electrophoresis Using Laser Induced Fluorescence Detection, Proceedings of 1996 Medical Defense Bioscience Review, Vol II (AD A321841), 751-756, 1997.

Manuscript in Preparation:

Lisinopril, a metalloprotease inhibitor, Delays Botulinum Toxin B Cleavage of VAMPII Measured on Capillary Electrophoresis. Karen E. Asermely, Clarence A. Broomfield, Brennie Hackley Jr., Frank J. Cann, Robert E. Sheridan, Harlan Shafer, Michael Adler. Journal of Neurochemistry.

Manuscript in Preparation:

Substance P Affects the cleavage of VAMPII by Botulinum Toxin B. Karen E. Asermely, Michael Adler, Clarence A. Broomfield. Journal of Biological Chemistry

(15) There are no patents or copyrights applications resulting from NRC Associateship Research.

(16) Current Position

Pharmacology Research Associateship Training (PRAT) Fellow at the National Institutes of Health for a 2 year appointment beginning on November 10, 1997.

Forwarding Address: Dr. Karen E. Asermely, National Institutes of Health, 36 Convent Drive, MSC-4090, Bethesda, Maryland 20892-4090. Telephone: 301-496-4690, Fax: 301-402-1748, e-mail: asermely@codon.nih.gov

(17) Appraisal of the Associateship Programs:

This program has been invaluable in advancing my career as a cholinergic pharmacologist. I learned protein biochemistry, neurotoxicology and new in-vitro techniques such as peptide synthesis, capillary electrophoresis and Edman sequencing which I need to investigate the role of proteins in neuronal function, in vitro. This experience has added a new area of research interests: neurotoxicology. I was able to collaborate closely with an internationally known protein biochemist, Clarence Broomfield, who practically served as a second mentor. Dr. Broomfield is on all three publications and desires to collaborate with me during my 2nd postdoctoral fellowship on mutual areas of interest. I had collaborated with a few others in the Neurotoxicology Branch at USAMRICD as evidenced by their contributions on these 3 papers also. Immersing myself in this special team of neuroscientists (headed by my research advisor, Dr. Michael Adler) working on botulinum toxins gave me a rare opportunity to grasp the field quickly, interact as a colleague and get up to speed on important scientific questions needed to be answered in the future. Dr. Michael Adler is also internationally known in the area of botulinum toxin. He is an outstanding scientist, a great person, but has fair management and communication skills. We have come up with research interests also which are of mutual interest and plan to collaborate after this NIH postdoctoral experience. My future plans are to go into a pharmacology department in academia/ or a Research Institute as a molecular neuropharmacologist/neurotoxicologist. The scientific relationships which I have formed with particularly, Dr. Brennie Hackley, Dr. Clarence Broomfield and Dr. Michael Adler will allow me to at the very least write competitive grants, hopefully get them funded and continue to work in this area of botulinum toxins and cholinergic transmission.

It is noteworthy to add that when I attended the Interagency Botulinum Meeting, the CDC and the scientists at the Univ. of Wisconsin were highly interested in the new methods and studies which have been accomplished in my tenure. This work is the first CE method which is successful and is more accurate (in my opinion) than existing HPLC methods in studying the actions of Botulinum toxins in vitro. I was approached by both research groups and told that they had a CE on order and wanted me to set up the new methods in their research groups. I am following up on this by trying to first publish my results and possibly consulting them or actually seeking a job at the Center for Disease Control(CDC) or Univ. of Wisconsin group after this NIH fellowship ends in 1999.

Karen E Asermely, Ph.D. 1-30-98.

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RECEIVED
JUL 24 1998
ASSOCIATESHIP PROGRAMS

RECEIVED
JUL

NATIONAL RESEARCH COUNCIL
Resident Research Associateships Programs Office
FINAL REPORT FORMAT

9449571

1) DATE July 16, 1998

2) NAME Dr. Apurba K. Bhattacharjee

3) NAME OF LABORATORY OR CENTER AND LOCATION Walter Reed Army Institute of Research, Wash, DC

4) DATES OF TENURE July 10, 1995 - July 9, 1998

5) TITLE OF RESEARCH PROPOSAL "Application of the Electronic Structures of Molecules to Drug Discovery"

6) NAME OF RESEARCH ADVISER Dr. Jean M. Karle

7) ARE YOU ON LEAVE FROM A PROFESSIONAL POST? Up until August 25, 1997
If so, list position or title and address. Associate Professor, Dept of Chemistry, Lady Keane College
Shillong 793001 (India)

8) PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE Nil

9) PROFESSIONAL TRAVEL DURING TENURE Enclosed
List location(s) and date(s) of travel to scientific meetings. List foreign meetings separately.

10) SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES Enclosed
List location(s) and date(s).

11) SUMMARY OF RESEARCH DURING TENURE Enclosed
List significant findings in concise form (100 words or less). Please do NOT use Greek letters or mathematical signs and symbols.

12) RESEARCH IN PROGRESS Enclosed

13) PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH Enclosed
Provide complete citation(s), including author(s), full name of journal, volume number, page number(s), and year of publication. **Please list separately:**
(a) Publications in peer-reviewed journals;
b) Books or book chapters; and,
() Manuscripts in preparation, manuscripts submitted.

14) PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES Enclosed
Provide complete reference with author(s), title, abstract or proceeding citation, and meeting name and location for international and domestic.

15) PATENT OR COPYRIGHT APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RESEARCH Nil

*16) FUTURE POSITION AND ADDRESS AND/OR FORWARDING ADDRESS
(Clearly indicate to which address you wish your tax statement mailed)
Research Scientist
Dept of Pharmacology
Div of Experimental Therapeutics, WRAIR
Washington, DC 20307-5100

17) APPRAISAL OF THE ASSOCIATESHIP PROGRAMS Enclosed
Comment on the usefulness of the Associateships Program to you, and include suggestions for improvements.

Associateships Programs Office use only
ID. Number_____ Copy To / Date_____ Cost Center_____

Regular mail
National Research Council
2101 Constitution Avenue NW
Associateships Programs (TJ 2114)
Washington, DC 20418

FAX
202 - 334 - 2759
EMAIL
_____@nas.edu

Express mail delivery
NRC Associateships Programs
1000 Thomas Jefferson Street NW
Suite 2114
Washington, DC 20007

9) PROFESIONAL TRAVEL DURING TENURE

National meetings:

1. *Experimental Biology 98,* San Francisco, CA, **April 18-22, 1998**

2. *46th Annual Meeting of the American Society of Tropical Medicine and Hygiene*, Orlando, Florida, **December 7-11, 1997.**

3. *37th Sanibel Symposium,* St. Augustine, FL, **March 1-7, 1997.**

4. *45th Annual Meeting of Am. Soc. Trop. Med. Hygiene,* at Baltimore, MD, **December 1-5, 1996.**

5. *211th ACS National Meeting* at New Orleans, LA, **March 24-28, 1996.**

Foreign meetings:

1. *Second Global Meet on Parasitic Diseases with a focus on Malaria,* Hyderabad, India, **August 18 - 22, 1997.**

2. *19th International Conference on Science & Technology* at New Delhi, **October 31-November1, 1996.**

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10) SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES

1. Uniformed Services University of the Health Sciences, Division of Clinical Pharmacology Seminar Series, Bethesda, MD 20814, **November 13, 1997.**

2. Howard University, Department of Chemistry, Washington, DC, **March 14, 1997.**

3. Walter Reed Army Institute of Research, Division of Experimental Therapeutics Journal Club Meeting, Silver Spring, MD 20910, **January 14, 1997.**

4. Uniformed Services University of the Health Sciences, Division of Clinical Pharmacology Seminar Series, Bethesda, MD 20814, **December 21, 1996.**

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11) SUMMARY OF RESEARCH DURING TENURE

(list of significant findings in concise form)

1. Correlated the stereoelectronic features of 52 4-quinoline carbinolamine antimalarials with their *in vivo* and *in vitro* activities for designing more efficacious antimalarial agents.

2. Validated an inexpensive methodology for predicting experimental neurotoxicity of 9 artemisinin compounds with a set of calculated molecular electronic discriminators that may be used to determine this complex biological activity with scope for application in a key emerging world health problem like malaria.

3. Identified specific electronic properties that define antimalarial activity of 8 cinchona alkaloids and the insect repellent potency of 31 DEET analogs that are associated with the potency of these compounds in order to better understand their molecular mechanism of action and to design more potent analogs.

****

12) RESEARCH IN PROGRESS

1. Designing of reversal agents against multidrug resistant malaria parasites and studying the mechanism of resistance, particularly the mechanism of P-glycoprotein/antimalarial drug interactions at the molecular level.

2. To assess the role of stereoelectronic properties of trioxane derivatives toward their experimental *in vitro* antimalarial efficacy in order to recommend structurally simple analogs that may be more soluble in water and less likely to have intrinsic neurotoxicity. Another objective is to obtain insights of the molecular level mechanism, specifically the role of the peroxide bond in antimalarial action.

3. To identify specific molecular electronic attributes that are associated with the potency of fifteen 8-aminoquinolines against drug resistant *Plasmodium falciparum* malaria.

4. Study of the role stereoelectronic properties on antivesicant property of thiol compounds.

5. To aid the design of more efficacious camptothecin analogs against malaria parasite and to better understand the mechanism at molecular level for the carbocation like transition state in the reaction pathway.

6. To study the effect of molecular electronic properties on phototoxicity of antimalarial agents.

7. "Docking" of potential antimalarial drugs to newly identified biochemical targets in malaria parasites.

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13) PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH

(a) *Publications in peer-reviewed journals:*

1. **Bhattacharjee, AK and JM Karle**. Functional Correlation of Molecular Electronic Properties with Potency of Synthetic Carbinol Antimalarial Agents, *Bioorg. & Med. Chem.,* **1998**, in press.

2. **Ma, D, Bhattacharjee, AK, Gupta, RK and Karle, JM.** Predicting mosquito repellent potency of DEET analogs from molecular electronic properties. *Am. J. Trop. Med. Hyg.,* revised, **1998.**

3. **Bhattacharjee, AK, and JM Karle.** Molecular electronic properties of a series of 4-quinolinecarbinolamines define antimalarial Activity Profile. *J. Med. Chem.,* 39: 4622-4629, **1996.**

4. **Karle, JM, and AK Bhattacharjee.** Trifluoromethyl group disorder in crystalline 2,8-bis(trifluoromethyl)-4-hydroxymethyl-quinoline. *J. Chem. Crystallography,* 26: 615-619, **1996.**

(b) *Manuscripts in preparation and submitted:*

1. **Karle, JM and AK Bhattacharjee**. Stereoelectronic Features of the Cinchona Alkaloids Determine their Differential Antimalarial Activity. *Pharm. Res.,* **1998**, submitted.

2. **Bhattacharjee, AK and JM Karle**. Stereoelectronic Predictors of Neurotoxicity Caused by Antimalarial Artemisinin Analogs. *Chem. Res. Toxicol.,* **1998,** in preparation.

3. **Bhattacharjee, AK, Ma, D, Karle, JM and Gupta, RK.** Molecular Similarity Analysis between Insect Juvenile Hormone Mimics and N,N-diethyl-3-methyl Benzamide (DEET) Analogs may Aid the Design of Novel Insect Repellents. *J. Med. Entomol.,* **1998**, in preparation.

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14) PRESENTATIONS AT SCIENTIFIC MEETINGS AND CONFERENCES

1. **Bhattacharjee, AK, and JM Karle.** Molecular electronic properties to aid the design of reversal agents for multidrug resistance of antimalarial mefloquine. *Experimental Biology 98,* San Francisco, CA, **April 18-22, 1998.**

2. **Karle, JM, and AK Bhattacharjee**. Using molecular electronic properties of 8-aminoquinolines to predict antimalarial potency. *215th ACS National Meeting* at Dallas, TX, **March 29 - April 2, 1998.**

3. **Bhattacharjee, AK, and JM Karle.** Prediction of *in vitro* neurotoxicity of the antimalarial artemisinin analogs using calculated molecular electronic properties. *46th Annual Meeting of the American Society of Tropical Medicine and Hygiene*, Orlando, Florida, **December 7-11, 1997**.

4. **Karle, JM, and AK Bhattacharjee**. Using molecular electronic properties of 4-quinolinecarbinolamines to predict antimalarial potency. *46th Annual Meeting of the American Society of Tropical Medicine and Hygiene,* Orlando, Florida, **December 7-11, 1997.**

5. **Ma, D, AK Bhattacharjee, JM Karle and RK Gupta.** Predicting mosquito repellent activity of DEET analogs from molecular electronic properties. *46th Annual Meeting of the American Society of Tropical Medicine and Hygiene,* Orlando, Florida, **December 7-11, 1997**.

6. **Bhattacharjee, AK, TR Kau and JM Karle.** Prediction of antimalarial potency using calculated molecular electronic properties. *Second Global Meet on Parasitic Diseases with a focus on Malaria,* Hyderabad, India, **August 18 - 22, 1997.**

7. **Karle, JM and AK Bhattacharjee.** Using molecular electronic properties of antimalarial artemisinin analogs to predict neurotoxicity. *Emerging Infections and Antimicrobial Resistance: Rational Approaches to Drug Design,* New Orleans, LA, **May 30 - June 1, 1997.**

8. **Bhattacharjee, AK and JM Karle.** The molecular electronic properties of antimalarial artemisinin analogs may predict in vitro neurotoxicity *37th Sanibel Symposium,* St. Augustine, FL, **March 1-7, 1997.**

9. **Karle, JM and AK Bhattacharje.** Electronic structure of the cinchona alkaloids predicts antimalarial activity. *45th Annual Meeting of Am. Soc. Trop. Med. Hygiene,* at Baltimore, MD, **December 1-5, 1996.**

10. **Bhattacharjee, AK and JM Karle.** The molecular electronic properties of structurally different synthetic aminoalcohols correlate with their antimalarial activities.
45th Annual Meeting of Am. Soc. Trop. Med. Hygiene at Baltimore, MD, **December 1-5, 1996.**

11. **Bhattacharjee, AK and JM Karle.** Application of molecular electronic properties in the designing of antimalarial drugs on a series of 4-quinolinecarbinolamines.
19th International Conference on Science & Technology at New Delhi, **October 31-November1, 1996.**

12. **Bhattacharjee, AK and JM Karle.** Molecular electronic properties may predict antimalarial activity.
211th ACS National Meeting at New Orleans, LA, **March 24-28, 1996.**

****

16) ADDRESS FOR MAILING TAX STATEMENT

Dr. Apurba K. Bhattacharjee
Apartment Number 111
2500 Wisconsin Avenue (NW)
Washington, DC 20007-4508
Tel: 202-944-2787

17) APPRAISAL OF THE ASSOCIATESHIP PROGRAMS

The associateship programs conducted by the National Research Council truly provides excellent opportunities for carrying out research on any topic of one's own choice if that matches with the interests of the sponsoring laboratory.

I found the program extremely useful for exchanging new ideas, approaches and techniques with my sponsoring laboratory where not only I could effectively contribute my experience and expertise but also could participate in the overall research climate of the laboratory. My research adviser (Dr. Jean M. Karle) had been extremely helpful in exchanging scientific ideas and very efficiently assisting me by providing all technical support and resources required for carrying out the research.

I am also very much thankful to the NRC staff for their remarkable efficiency and helping attitude. I never had any problem with the administration during my entire tenureship. As far as suggestions for improvements are concerned, I wonder (i) whether the Form 1042S for the Nonresident aliens can be expedited to the month of January so as to enable the Associate a timely refund from the IRS and (ii) whether in addition to NAT, a couple of other competitive travel agents can be enlisted for the benefit of travel requirements of the Associates as I have found the NAT airline tickets to be consistently at a higher cost than can be obtained otherwise.

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960590

National Research Council
Associateship Programs
2101 Constitution Ave, NW TJ 2114 (202/334-2759) FAX
Washington, DC 20418

NRC Fellowship Final Report

Signatures:
Advisor, Dr. Michael Parker: Michael D Parker Date: 11-12-97
LPR Dr. Carrol Linden: Carrol Linden Date: 11/12/97

(1) November 11, 1997

(2) Bruce Crise Bruce Crise 11-12-97
NRC Associateship I.D. Number:_____

(3) Name of Laboratory:
U. S. Army Medical Research Institute of Infectious Diseases,
Fort Detrick, MD 21702

(4) Dates of Tenure:
November 14, 1996 to October 28, 1997

(5) Title of Research Proposal:
Development of Vaccines for Serotype IE Strains of Venezuelan Equine Encephalitis Virus

(6) Name of Research Advisor:
Michael D. Parker

(7) Not on leave from a professional post

(8) No professional offices held

(9) Professional Travel:
American Society of Virology annual meeting
Bozeman, Montana
7-19-97 to 1-23-97

(10) Lectures Delivered:
Guest Lecturer in graduate level virology course
Hood College
Frederick, MD
9/23/97, 9/30/97, and 10/7/97

(11) Summary of Research During Tenure:

Please see attached

(12) Research in Progress:
A. Continued vaccine studies with VEE IE
B. Molecular characterization of C-type Bunyaviruses
C. Analysis of cross-protection between Filoviruses
D. Characterization of factors modulating Alphavirus replicons

(13) No presentations at scientific meetings

(14) No publications or papers

(15) Patent Applications Resulting From NRC Associateship Research:
Patent Submission: Parker, M.D., J. F. Smith, and B. Crise. 1997. Full length cDNA of Western Equine Encephalitis Virus and Venezuelan Equine Encephalitis IE variant and uses thereof. Submitted.

(16) Future Position and Address:
Microbiologist
Virology Division
USAMRIID
1425 Porter Street
Ft. Detrick, MD 21702

(17) Appraisal of the Associateship Programs:
Please see attached

NRC Fellowship Final Report, continuation

(11) Summary of Research During Tenure:

During my tenure as an member of the NRC Associateship Program I completed construction of molecular clones of serotype IE strains of Venezuelan Equine Encephalitis Virus (VEE IE). One of these clones was identical to the biological isolate of VEE IE as assayed by in vitro growth characteristics and virulence in rodents. This virulent clone was then used as a template for vaccine production. Attenuating mutations were introduced into the clone by deleting regions of the structural genes of the virus. These attenuated clones were tested in animals for virulence and their ability to protect against lethal challenge from inoculation with the virulent biological isolate of VEE IE. One candidate clone was chosen for further testing and development as a vaccine that will have potential as both a human and veterinary vaccine. Other projects listed under item 12.

(17) Appraisal of the Associateship Programs:

My tenure as a National Research Council fellow has been great opportunity for me to continue my training and development as a scientist. Through the Associateship Program I was able to gain access to the unique laboratory facilities at USAMRIID. USAMRIID was an excellent scientific environment in which I was able to work as an integrated member of the research staff on challenging and thought provoking projects.

As a member of the NRC Associateship Program I was supported by the personnel at USAMRIID from both the administrative and scientific arenas. In particular, the past and present Virology Division chiefs, Jerry Jennings, William Pratt and George Korch were supportive of my work. Most importantly, my scientific interactions with Michael Parker and Jonathan Smith have contributed greatly to my work and training. I hold their qualities as scientists and mentors in the highest regard.

9605830

NRC FINAL REPORT

1) **DATE**: NOVEMBER 20, 1998

2) **NAME**: Dr. Rina Das

3) **NAME OF LABORATORY OR CENTER**: Division of pathology, Walter Reed Army Inst. Of Research, Washington D.C. 20307-5100

4) **DATES OF TENURE**: Oct 1996-98

5) **TITLES OF RESEARCH PROPOSAL**: SIGNAL TRANSDUCTION PATHWAYS FOR BIOACTIVE LIPIDS IN BREAST CANCER

6) **NAME OF RESEARCH ADVISOR**: Dr. Marti Jett

7) **ARE YOU ON LEAVE FROM A PROFESSIONAL POST**? NO

8) **PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE**:

Members of: ASCB, AACR, AAAS

9) **PROFESSIONAL TRAVEL DURING TENURE**

- Keystone symposia, in Keystone, Colorado, March 1997
- 17th International Congress of Biochemistry and Mol. Biol., San Francisco, CA, Aug1997
- ASBMB annual meeting, Washington D.C., 1998
- 21st Army Science Conference, Norfolk, VA, 1998;

10) **SEMINARS OR LECTURES DELIVERED**: Department Seminars

11) **SUMMARY OF RESEARCH DURING TENURE**: We analyzed the role of raf, MEK, MAP Kinase and 5-LO in the signaling of IGF-I in human breast cancer cells /(578T). We have determined that raf and MEK were both phosphorylated within 1 minute of IGF-1 treatment, reaching its peak by five minutes. MAP Kinase was also phosphorylated after five minutes of exposure to IGF-1. Rapid tyrosine phosphorylation of 5-LO was observed within 1 minute of IGF-1 stimulation. These results so far suggest that both signaling pathways are activated in response to IGF-1 in human breast cancer cells. In order to resolve the exact role of each of these kinases and establish a possible cross talk between the signaling pathways, we used several known inhibitors of these pathways. By inhibiting each of the pathways at different points, we should be able to pinpoint the exact relationship between the two cellular signaling pathways. The forthcoming results might suggest that the two signaling pathways might be connected in some fashion.

We have also analyzed the role of fatty acid binding proteins in breast cancer. We have measured the levels of these proteins in normal and cancer cells and have observed distinct pattern of these fatty acid binding proteins that can act as biomarkers of cancer.

12) **RESEARCH IN PROGRESS**: The research has evolved into a different project that is in progress at the same facility under the same mentor.

13) **PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP**:

a) Manuscript under preparation:
R. Das, N. Kodsi and M. Jett CROSS TALK BETWEEN MAP KINASE PATHWAY AND ARACHIDONIC ACID PATHWAY IN THE SIGNALING CASCADE OF IGF-1 IN BREAST CANCER CELLS.

14) **PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES**:

- **Das, R.**, Terry-Koroma, B. and Jett, M. (1998) Characterization of fatty acid binding proteins in prostate cancer cells. In Annual ASBMB meeting, The Faseb Journal, vol 12, Abst# 395.
- You, Y., Zhang, X., **Das, R.** and Jett, M. (1997) Cell cycle effects of mammary derived growth inhibitor in MDGI gene transfected breast cancer cells. In 37th Annual Meeting of American Society for Cell Biology. Abst # 88.
- **Das, R.,** Kodsi, N. and Jett, M. (1997) Cross talk between the MAP kinase and the Arachidonic Acid pathway in signal transduction of growth factor in breast cancer cells. FASEB Journal 10: 2852. ASBMB meeting San Francisco 1997.
- X. Zhang, **R. Das** and M. Jett (1997) Meeting Abstract in "Frontiers in Breast Cancer Res. Diagnosis and Therapy".

15) **PATENT APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RES.**: Yes
M. Jett, **R. Das** and R Neill. Using the measurement of levels of fatty acid binding proteins as predictors of stage or aggressiveness of breast and prostate cancer (Pending).

16) **FUTURE POSITION TITLE AND STATUS**: Sr. Scientist at same lab at WRAIR

17) **FORWARDING ADDRESS**: Rina Das, 2613 Northrup Drive, Rockville, MD-20850.

18) **APPRAISAL OF THE ASSOCIATESHIP PROGRAMS**: It is a good program to get training after PhD. and helps an individual to establish in their field of interest. The health insurance coverage could be better.

93A0860

FINAL REPORT
NATIONAL RESEARCH COUNCIL

1 **DATE** October 01, 1997

2.. **NAME:** **Michael Okechukwu Eze**

NRC Associateship I.D. Number: 97 . 15 . 10 . 09

3. **NAME OF LABORATORY OR CENTER AND LOCATION:** WRAIR, Washington, DC.

4. **DATES OF TENURE:** **October 03, 1994 - October 02, 1997.**

5. **TITLE OF RESEARCH PROPOSAL:** Replication of virulent and avirulent brucellae in human monocytes and in mice.

6. **NAME OF RESEARCH ADVICER:** DR. D. L. Hoover, COL. MC.

7. **ARE YOU ON LEAVE FROM A PROFESSIONAL POST?:** Yes

If so, list position or title : Senior Lecturer

Address: Department of Biochemistry, University of Nigeria, Nsukks, Nigeria.

8. **PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE:** None

9. **PROFESSIONAL TRAVEL DURING TENURE:**

List location(s) and date(s) of travel to scientific meetings. List foreign meetings separately

Meetings within USA

	Location	Scientific Meetings	Dates
i.	Chicago, Illinois	47th Annual Brucellosis Conference	Nov. 11-13, 1994
ii.	Chicago, Illinois	48th Annual Brucellosis Conference	Nov. 10-12, 1995
iii.	New Orleans, Louisiana	96th General Meeting, American Society for Microbiology (ASM)	May 19-23, 1996
iv.	Chicago, Illinois	49th Annual Brucellosis Conference	Nov. 08- 10 , 1996

Foreign Meeting

Location	Scientific Meeting	Dates
Quebec City, P. Q., Canada	40th Annual Meeting of the Canadian Federation of Biological Societies (CFBS)	June 17-22, 1997

10. **SEMINARS OR LECTURES DELIVERED AT UNIVERSIITIES AND/OR INSTITUTES:**

List location(s) and date(s): None

11. **SUMMARY OF RESEARCH DURING TENURE:**

List significant findings in concise form (100 words or less). Please do not use Greek letters or mathematical signs and symbols.

We studied the growth of brucellae in mouse peritoneal macrophages and human monocytes in culture, and the role of gamma-interferon in murine anti-*Brucella* immunity. Gamma-interferon activates cultured mouse macrophages to produce nitric oxide, and to inhibit intracellular replication of *Brucella*. Infection in mice causes influx of macrophages to the spleen. Macrophages from gamma-interferon-depleted *Brucella*-infected mice have a greater bacterial burden than control mice. They however produce as much nitric oxide as controls when adequately stimulated

in culture. This emphasizes that gamma-interferon is important in nitric oxide-mediated macrophage anti-*Brucella*. immune responses.

12. RESEARCH IN PROGRESS:

In the course of these studies, we have generated a large number of samples which are awaiting analyses to yield further insight into the mechanism of the immune response of the host against *Brucella* infection. Samples of culture supernatants will be analyzed by ELISA for cytokines, and RNA samples by reverse transcription and polymerase chain reaction for cytokine message and for message for inducible nitric oxide synthase.

13. PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES:

i. "Effects of opsonization uptake and growth of Brucella melitensis 16M in mouse peritoneal macrophages" [co-authors: M. Eze, R. Crawford, J. Drabick, T. Hadfield, A. Bhattacharjee, R. Warren, and D. Hoover]. Abstract #D-88, Session 203, page 257 of Book of Abstracts. Poster presented at the 96th General Meeting of the American Society for Microbiology, New Orleans, Louisiana, USA, May 19-23, 1996.

ii. "Serum opsonization and nitric oxide in macrophage clearance of Brucella." [co-authors: M. Eze, R. Crawford, J. Drabick, T. Hadfield, A. Bhattacharjee, R. Warren, and D. Hoover]. Scientific presentation on April 15, 1996 to the Brucella Program Review panel, USAMRMC Biological Defence Research Program, held at Walter Reed Army Institute of Research, Washington, DC.

iii. "Uptake and survival of opsonized Brucella melitensis 16M in murine peritoneal macrophages." [co-authors: M. Eze, R. Crawford, J. Drabick, T. Hadfield, A. Bhattacharjee, R. Warren, and D. Hoover]. Scientic presentation at the 49th Annual Brucellosis Conference, Chicago, Illinois, november 08-10, 1996.

iv. "Nitric oxide in the control of serum-opsonized Brucella bymurine macrophages." [co-authors: M. Eze, R. Crawford, J. Drabick, T. Hadfield, A. Bhattacharjee, R. Warren, and D. Hoover]. Abstract, Poster #143. Canadian Federation of Biological Societies 40th Annual Meeting, Quebec City, Quebec, Canada, June 18-81, 1997.

14. PUBLICATIONS AND PAPERS RESULTING FROM FROM NRC ASSOCIATESHIP RESEARCH:

(a) Publications in peer-reviewed journals: None yet
(b) Books or Book chapters: None
(c)Manuscripts in preparation:

i. M. Eze, R. Crawford, J. Drabick, T. Hadfield, A. Bhattacharjee, R. Warren, and D. Hoover. "Effects of opsonization and gamma-interferon on growth of Brucella melitensis 16M in mouse peritoneal macrophages."

ii. M. Eze, R. Crawford, J. Drabick, T. Hadfield, A. Bhattacharjee, R. Warren, and D. Hoover. " Nitric oxide production and growth of Brucella melitensis 16M in macrophages from gamma-interferon-depleted mice.

15. PATENT OR COPYWRITE APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RESEARCH:

none.

16. FUTURE POSITION AND ADDRESS AND/OR FORWARDING ADDRESS:

Forwarding Address:

312 - 4810 Millwoods Road South Edmonton Alberta, CANADA T6L 5N9
Telephone: (403) 450-9530
FAX: (403) 431-8731

17. APPRAISAL OF THE ASSOCIATESHIP PROGRAMS

Comment on your program and its usefulness to you. Suggest improvements in the overall Associateship Programs.

I have derived immense benefit from this program. It has enriched me professionally and I have interacted with real fine people in the process of executing my research. I do hope and pray that the system benefitted as much as I got from it, and more. May I at this point express my sincere appreciation for the opportunity to participate in the program.

In my view, there is no apparent weakness in the program. I suggest that efforts be made to keep the standards already in place.

NRC Final Report

1. Date:26 June 1998
2. Name: Michal Fried
3. Name of laboratory or center and location: US Army Medical Research Unit-Kisumu, Kenya. Dept. of Immunology, Walter Reed Army Institute of Research, Washington, DC.
4. Dates of Tenure: 10 January 1995-10 July 1998
5. Title of research proposal: Identification of placental ligands and cytokines involved in maternal malaria.
6. Name of research adviser: Patrick Duffy
7. Are you on leave from a professional post?
   No
8. Professional society offices held during tenure
   None
9. Professional Travel during tenure:
   ASTMH November 1995, San Antonio, Texas
   ASCB, December 1995, Washington, DC
   ASTMH December 1996, Baltimore, MD
   18th African Health Sciences Congress, April 1997, Cape Town, South Africa
   ASTMH December 1997, Orelando, Florida
10. Seminars or lectures delivered at universitties and/or institute :
    NIH, November 1995
    WRAIR, November 1995
    ILRI-Nairobi, September 1996
    WRAIR, December 1996
    Texas A&M, March 1998
11. Summary of research during tenure.

Women become highly susceptible to malaria infection during their pregnancy, and this susceptibility is greatest during their first pregnancy. Because pregnant women who reside in endemic areas had acquired anti-parasite and anti-disease immunity comparable to their non-pregnant counterparts, scientists hypothesized that the susceptibility resulted from pregnancy-related immunosuppression. However, because the high susceptibility to malaria infection is principally associated with first pregnancies, gestational immunosuppression may be an insufficient explanation of pathogenesis. Taking a different approach, I described the molecular basis for maternal malaria: a distinct subpopulation of parasites binds to chondroitin sulfate A (CSA) and not other receptors which commonly mediate parasite adhesion. During first pregnancy, placental syncytiotrophoblasts present CSA as a new substrate for parasite adhesion, selecting CSA-binding parasites to sequester and propagate. In further studies, I have shown that women develop specific humoral immunity over successive pregnancies which inhibits parasite binding to the placental cell surface. This work suggests that anti-adhesion antibodies confer protection, and that naturally occurring humoral immunity to a parasite protein(s) is protective and can be used as an effective strategy to develop a vaccine. Testing sera from Thailand and Malawi, I demonstrated that sera from multigravid women but not primigravid women inhibited the adhesion of Kenyan parasite isolates to CSA, that parasites collected from Thai pregnant women have the same binding phenotype as parasites collected from Kenyan pregnant women, and that sera obtained from Kenyan parturients inhibited the adhesion Thai parasite isolates to the same degree that these sera inhibited Kenyan isolates. These data demonstrate that the development of a protective anti-adhesion humoral immune

response is a global phenomenon. I have separately shown that malaria infection shifts the immune environment in pregnant women from Th2 type (protective immunity during pregnancy) to a Th1 type of immune response, and this change is associated with low birthweight infants and anemia in the mother.

12. Research in progress:
Currently I am focusing on identifying and cloning the gene encoding the chondroitin sulfate A-binding protein(s).

13. Publications and papers resulting from NRC associateship research.
    1. Fried M, PE Duffy. 1996. Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. Science 272:1502-1504.
    2. Fried M, RO Muga, AO Misore, PE Duffy. 1998. Malaria elicits type 1 cytokines in the human placenta: interferon-γ and tumor necrosis factor-α associated with pregnancy outcomes. J Immunol. 160:2523-2530.
    3. Fried M, PE Duffy. 1998. Maternal malaria and parasite adhesion. J. Mol. Med. 76:162-171.
    4. Fried M, F Nosten, A Brockman, BJ Brabin, PE Duffy. 1998. Anti-adhesion antibodies in women resistant to malaria of pregnancy. Submitted for publication.
    5. Fried M, PE Duffy eds. 1998. Maternal malaria. Publisher Landes Bioscience. In preparation.

14. Presentation at scientific meetings or conferences:
    1. Fried M, PE Duffy. ASTMH 1995. Chondroitin sulfate A is the adhesion receptor for *Plasmodium falciparum* -infected erythrocytes in the human placenta.
    2. Fried M, PE Duffy. ASTMH 1995. Cytokine secretion in placentas obtained from malaria-infected women.
    3. Fried M, PE Duffy. ASCB 1995. Chondroitin sulfate A is the adhesion receptor for *Plasmodium falciparum* -infected erythrocytes in the human placenta. (This abstract was chosen among 2,675 abstracts as one of fifteen that appeared in the Press Book).
    4. Fried M, PE Duffy. ASTMH 1996. Placental responses to malaria infection.
    5. Fried M, PE Duffy. 18 African Health Conference, 1997. Placental responses to malaria infection.
    6. Fried M, PE Duffy. ASTMH 1997. Inhibition of placental parasite adhesion

15. Patent or copyright applications resulting from NRC associateship research
None

16. Future position and address
Staff Scientist
Dept. of Immunology
Bldg. 40, Rm.2028
Walter Reed Army Institute of Research
14th and Dahlia St.
Washington, DC 20307-5100

17. The NRC associate program gave me an opportunity to progress professionally and personally. I was able to develop an independent research program in the area of my interest, and to work in Africa, studying malaria parasites in a region where the disease occurs naturally. I would also like to express my appreciation for my supervisor, Dr. Patrick Duffy, whose example as a brilliant scientist and a leader was an inspiration for my own successes.

Sara Rockman 8 Jul 98

NATIONAL RESEARCH COUNCIL

FINAL REPORT

(1) DATE: March 30, 1998

(2) NAME: **Vera de Souza Gouvea**

(3) NRC ID: 948260

(4) LABORATORY: Department of Virus Diseases
Walter Reed Army Institute of Research
14th & Dahlia St. Bldg 40, Washington DC 20307

(5) DATES OF TENURE: October 5, 1994 - 28 February, 1998

(6) TITLE OF RESEARCH: Molecular and Antigenic Characterization of Putative Hepatitis E Virus Variants

(7) NAME OF ADVISER: Bruce L. Innis

(8) ON LEAVE FROM A PROFESSIONAL POST? No

(9) PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE:
American Society for Virology
American Society for Microbiology
American Society of Tropical Medicine and Hygiene
Brazilian Society of Virology

(10) PROFESSIONAL TRAVEL DURING TENURE:

July 1995 - American Society for Virology, 14th Annual Meeting, Austin, Texas.
November 1995 - American Society of Tropical Medicine and Hygiene, 1995 Annual Meeting, San Antonio, Texas.
August 1996 - Xth International Congress of Virology, Jerusalem, Israel.
October 1996 - SmithKline Beecham and WRAIR Consortium on the Medical Need for a HEV Vaccine, Kathmandu, Nepal, and AFRIMS, Bangkok, Thailand. (sponsored by SKB and WRAIR)
November 1996 - Sociedade Brasileira de Virologia, Biannual Meeting, São Lourenço, MG, Brazil. (sponsored by SBV)
July 1997 - American Society for Virology, 15th Annual Meeting, Bozman, Montana.
December 1997 - American Society of Tropical Medicine and Hygiene, 1997 Annual Meeting, Coronado Springs, Florida.

(11) SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES:

"PCR-based diagnostic for hepatitis E", presented at the SmithKline Beecham Biologics and WRAIR consortium to evaluate the medical need for a HEV vaccine, Kathmandu, Nepal, and at AFRIMS, Bangkok, Thailand, October 1996.

"Hepatitis E", presented at the Hepatitis Section of the VIII National Meeting of the Brazilian Society for Virology, São Lourenço, MG, Brazil, Novemver 1996.

(12) SUMMARY OF RESEARCH DURING TENURE:

Information on the genomic and antigenic diversity of HEV strains is essential to evaluate vaccine efficacy. Attempts to recover and characterize HEV variants led to a detailed evaluation of procedures to recover viral RNA from clinical specimens, produce genomic cDNA copies and rapidly identify the virus isolate. Thus, a very sensitive and specific multi-site nested RT-PCR followed by restriction endonuclease analysis was developed, which allowed the rapid classification of all known HEV isolates into genotypes and subgenotypes precluding the need for cloning and sequencing (ref.2, 3, A2, A3). The utility of the method was demonstrated in the first studies of HEV isolates recovered from Nepal (ref.4,A4) and during a hepatitis outbreak among soldiers deployed to Haiti (ref.1, A1). The first complete sequence of a Nepali isolate (TK15/92) was accomplished and its genome was compared to the genomes of all other known HEV isolates (ref. 5, submitted)

(13) RESEARCH IN PROGRESS:

A cDNA library of clones from isolate TK15/92 was constructed and is being assembled into a single clone representing the full-length viral genome. This clone would be valuable for insertion into a vector system for expression and transfection for assessment of infectivity in cells or monkey liver.

(14) PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH:

(a) Publications in peer-review journals:

1. Drabick, J.J., Gambel, J.M., Gouvea, V.S., Caudill, J.D., Sun, W., Hoke Jr., C.H. & Innis, B.L. A cluster of acute hepatitis E infection in UN Bangladeshi peacekeepers in Haiti. Am. J. Trop. Med. Hyg. 57, 449-454, 1977
2. Gouvea, V., Cohen, S.J, Santos, N., Myint, K.S.A., Hoke Jr., C.H. & Innis, B.L. Identification of hepatitis E virus in clinical specimens: amplification of hydroxyapatite-purified virus RNA and restriction endonuclease analysis. J. Virol. Methods 69, 53-61, 1997
3. Gouvea, V, Hoke Jr., C, and Innis, B.I. Genotyping of hepatitis E virus by restriction endonuclease analysis. J. Virol. Methods, 70, 71-78, 1998
4. Gouvea, V., Snellings, N., Cohen, S.J., Warren, R.L., Myint, K.S.A., Shrestha, M.P., Vaughn, D.W., Hoke Jr., C. and Innis, B.L. Hepatitis E virus in Nepal: similarities with the Burmese

and Indian variants. Virus Res. 52: 87-96, 1997

(b) Books or book chapters: None

(c) Manuscripts in preparation or submitted:

5. Gouvea V, Snellings N, Popek MJ, Longer CF, and Innis BL. 1998. Hepatitis E virus: complete genome sequence and phylogenetic analysis of a Nepali isolate. Virus Res. (submitted)

6. Gouvea et al, 1998. Evidence for HEV-like agent in swine in USA (manuscript in preparation)

(15) PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES:

A1. Gouvea, V., Innis, B.L., Drabick, J., Gambel, J., Seriwatana, J., Hoke, C.H. Hepatitis E in Bangladeshi soldiers serving in the United Nations mission to Haiti (UNMIH). Abstract of the Xth International Congress of Virology, Jerusalem, Israel, PW54-15, p. 241, 1996

A2. Gouvea, V., Innis, B.L., Drabick, J., Gambel, J., Seriwatana, J., Caudill, J., Hoke Jr., C.H. Hepatitis E in Bangladeshi soldiers deployed to Haiti. Abstr. American Society for Virology, 15th Annual Meeting, London, Ontario, Canada, P2-1, p. 165, 1996

A3. Gouvea, V., Cohen, S.J, Santos, N., Myint, K.S.A., Hoke Jr., C.H. & Innis, B.L. Detection and genotyping of hepatitis E virus (HEV) in clinical specimens. Am. J. Trop. Med. Hyg. 57 (3, suppl), abstr. 330, pp. 213, 1997.

A4. Gouvea, V., Snellings, N., Cohen, S.J., Warren, R.L., Myint,K.S.A., Shrestha, M.P., Vaughn, D.W., Hoke Jr., C. and Innis, B.L. Hepatitis E virus in Nepal: similarities with the Burmese and Indian variants. Am. J. Trop. Med. Hyg. 57 (3, suppl), abstr. 329, pp. 212, 1997.

(16) PATENT OR COPYRIGHT APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RESEARCH: None

(17) FUTURE POSITION AND ADDRESS AND/OR FORWARDING ADDRESS: (FOR TAX STATEMENT MAILING)

Present home address: 1220 N. Pierce St. apt 701,
Arlington, VA22209
Tel/Fax: 703-522-8431

(18) APPRAISAL OF THE ASSOCIATESHIP PROGRAMS:

I found the NRC Associateship Program exceptional. I received prompt and friendly assistence and support from the highly qualified and efficient NRC staff. The easiness of communication and lack of burocracy at NRC allowed me to focus on the research and to accomplish projects at WRAIR, despite major personal difficulties.

FINAL REPORT

James Higgins, NRC Fellow
December 2, 1997

Location: USAMRIID, Diagnostic Systems Division

Tenure: January 15, 1997-December 22, 1997

Title of research proposal:" Molecular detection of bacterial pathogens using a novel 5' nuclease assay"

Research Advisor: M. Sofi Ibrahim

Professional travel during tenure: Seven Springs Resort, Champion, PA, Sept. 21-24, 1997, and Walt Disney World, Orlando, FL, December 7-11, 1997.

Seminars at Universities and/or Institutes: USDA Immunology and Disease Resistance Laboratory, Beltsville, MD June 19, 1997, and Diagnostic Systems Division, USAMRIID, August 15, 1997

Summary of research during tenure:

TaqMan 5' nuclease PCR assays (Perkin Elmer, Foster City, CA) were optimized, and clinical testing begun, for the bacterial pathogens *Brucella abortus*, *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*. For the assays as a whole, detection limits in the 1-10pg range were observed; probes demonstrated genus or species-specificity, as designed; and the assays were capable of detecting bacteria in infected animal and human tissues and/or blood. The assays worked when samples were processed using a simple filter-paper based protocol. The *Yersinia*, *Bacillus*, and *Francisella* assays were valid when performed on prototype miniature thermal cycler instruments.

Research in progress: Clinical specimens (insect vector, animal, and human tissues) provided by various collaborating investigators are still being assayed for *Yersinia*, *Brucella*, and *Francisella*. Performance evaluation of the assays on prototype miniature thermal cyclers is ongoing.

Presentations at scientific meetings or conferences: see attached sheet.

Manuscripts submitted and/or in preparation: "A 5' nuclease PCR assay to detect *Yersinia pestis*", submitted to The Journal of Clinical Microbiology, Nov. 30, 1997; in preparation, "Detection of *Brucella* spp. using a novel 5' nuclease PCR assay", and "Detection of *Francisella tularensis* using a novel 5' nuclease PCR assay".

Future position and forwarding address (as of Dec. 22, 1997):

James Higgins
USDA-ARS
Immunology and Disease Resistance Laboratory
BARC-EAST, bldg. 1040
Beltsville, MD 20705

Appraisal of the Associateship Programs: I enjoyed my tenure working at USAMRIID and, if I had not been offered the job at USDA, I would have either continued my tenure, or applied for a GS level position at the Institute.

I do have one suggestion: the requirement of five years post-doctoral status before one can qualify for the Senior Associateship-level salary needs to be changed, to three years. In the current job market for researchers, it is not at all unusual for individuals to spend three to four years in a particular postdoc position, trying to accumulate enough publications and grant applications to be a competitive applicant for positions in academe or government/industry. By this time they may well be earning salaries close to, or in excess of, that offered for beginning NRC Associates. By providing three or four-year postdocs with Senior Associate-level salaries, NRC fellowships will be more attractive opportunities for those postdoctoral researchers who are "holding out", as it were, for that assistant professor position "just around the corner"....

American Society of Tropical Medicine and Hygiene, Orlando, FL, Dec. 7-11, 1997

162 297 DETECTION OF HUMAN PATHOGENIC *BRUCELLA* SPECIES USING A NOVEL 5' NUCLEASE ASSAY. Higgins JA*, Hadfield T, Hilyard EJ, Ezzell JW, and Ibrahim MS. Diagnostic Systems Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Armed Forces Institute of Pathology, Washington, DC.

The 5' nuclease PCR assay detects PCR amplicons by exploiting the proclivity of *taq* polymerase to cleave the 5' end of an oligonucleotide probe (Taqman™) bound to a DNA target.The activity of the probe can be monitored and quantified with the ABI 7700 model Sequence Detector (PE Applied Biosystems). This device provides real-time analysis of PCR amplification reactions, and obviates the need to use agarose gels to determine if amplification occurred. We have developed a 5' nuclease PCR assay for *Brucella* spp. The assay can detect all serotypes of *Brucella abortus*, several strains of *Brucella melitensis*, and *Brucella suis*. The assay takes only 2 hr to complete, is genus-specific, and can detect little as 10 pg of bacterial DNA. The *Brucella* 5' nuclease PCR assay provides sensitivity and specificity equal to that of conventional PCR, in a labor-saving, rapid diagnostic format. We are also developing 5' nuclease PCR assays for other species of pathogenic bacteria. Our ultimate goal is to provide field-operative molecular diagnostic capabilities, to those areas where more elaborate facilities are lacking.

American Society of Rickettsiologists, Champion, PA, Sept. 21-24, 1997

59. NEW METHODS FOR RAPID DETECTION OF BACTERIAL PATHOGENS. **James A. Higgins* , M. Sofi Ibrahim, John Ezzell, and Erik A. Henchal**. *Diagnostic Systems Division, USAMRIID, Ft. Detrick, MD 21702-5011.*

The use of the polymerase chain reaction for the detection of infectious disease pathogens has revolutionized diagnostic microbiology. Newer techniques representative of "second generation" PCR technology are now becoming available.
One such technique is the 5' nuclease fluorogenic PCR assay (5NA), which exploits the proclivity of *taq* polymerase to cleave a fluorogenic probe bound to a target DNA strand. We have used the fluorogenic PCR assay and the ABI 7700 Model Sequence Detectort (Applied Biosystems Division, Perkin Elmer) to obtain real-time, rapid detection of several bacterial pathogens, including *Brucella* spp, *Yersinia pestis*, and *Francisella tularensis*. The system allows quantitative analysis of up to 96 samples in less than 3 hours. The assays we developed are highly specific and can detect picogram amounts of DNA. As part of our long-term goal of applying 5NA technology to situations where elaborate laboratory facilities may be lacking, assays will be adapted to use of the Miniature Analytical Thermal Cycler Instrument (Lawrence Livermore Laboratories). Ongoing implementation of these computer chip-based technologies to pathogen detection will be discussed.

December 2, 1997

Dr. Judy Nyquist
Deputy Director and Program Administrator
NRC Associateship Programs
2101 Constitution Avenue
Washington, DC

Dear Dr. Nyquist:

Enclosed is the Final Report of my Research Associateship, in the suggested format. Please note that it is rather premature, in that the projects I have started here at USAMRIID will be ongoing for at least another several months. Because of the proximity between USAMRIID and USDA-Beltsville, I intend to continue to use RIID facilities until my bacterial projects are completed, and perhaps to initiate other collaborative projects after that (?) In any event, I will provide updates to your office on the status of manuscripts in preparation, etc. that were begun during my Associateship tenure.

Sincerely,

James A Higgins

James Higgins
DSD-USAMRIID
1425 Porter St
Frederick, MD 21702

encl: Final Report

cc: Dr Carol Linden
Dr M. Sofi Ibrahim

Final Report

1. **Date:** December 31, 1997
2. **Name:** Dr. Jay W. Hooper
3. **Laboratory:** USAMRIID
4. **Dates of tenure:** July 1995-December 1997
5. **Title of research proposal:** Development of an Infectious Clone of Hantaan Virus.
6. **Name of research adviser:** Dr. Connie Schmaljohn
7. **Are you on leave from a professional post?** No
8. **Professional society offices held during tenure:** none
9. **Professional travel during tenure:**
   I have traveled to the ASV meeting in Bozman, Montana (July 97). I have attended two meetings in foreign countries: the American Society for Virology (ASV) annual meeting in London, Ontario (July 96), and the Tenth International Conference on Negative Strand Viruses in Dublin, Ireland (September 97).
10. **Seminars or lectures delivered at Universities and/or institutes:** none

11. **Summary of research during tenure:**
    I've developed three systems that may ultimately allow reverse genetic analysis of Hantaan virus (HTN). The first system involves an infectious clone strategy requiring cotransfection of 7 plasmids into animal cells followed by plaque assay to detect generated virus. The second system involves a rescue strategy using a synthetic HTN gene containing a selectable mutation to rescue virus. The third system involves a reporter plasmid designed to detect HTN polymerase activity. I've completed the plasmid constructs and have worked out the procedures required for each system. Experiments designed to trouble-shoot these systems are in progress. I've also generated and characterized a panel of HTN thermostable variants to serve as substrate for future reverse genetics experiments.

12. **Research in progress:**
    A. Further attempts to generate Hantaan virus from the plasmid constructs I've developed.
    B. Further attempts to rescue an engineered Hantaan virus containing a gene derived from a plasmid containing the M antigenome tagged with a selectable (neutralizing antibody escape mutation) marker.
    C. Further attempts to detect Hantaan virus polymerase activity using a reporter construct comprised of a luciferase gene (antisense orientation) flanked by the Hantaan M gene nontranslated regions (genomic sense).

13. **Presentations at scientific meetings or conferences:**
    1. J.W. Hooper and C.S. Schmaljohn. HANTAAN VIRUS THERMOSTABLE VARIANTS. 10th International Conference on Negative Strand Viruses. September, 1997. Dublin, Ireland.

2. J.W. Hooper, K. Anderson and C.S. Schmaljohn. ATTEMPTS TO GENERATE HANTAAN VIRUS INFECTIOUS CLONE USING NAKED-DNA APPROACH.Submitted to The 4th International Conference on HFRS and Hantaviruses. Atlanta, Georgia. March 1998.

14. Publications and papers resulting from NRC Associateship research:

J.W. Hooper and C.S. Schmaljohn. Hantaan Virus Thermostable Variants. Manuscript in preparation.

15. Future position and address and forwarding address:

Microbiologist. Virology Division. United States Army Medical Research Institute of Infectious Diseases. Ft. Detrick, Maryland 21702.

16. Appraisal of the associateship programs:

I've enjoyed my tenure as a NRC associate here at USAMRIID. This position has allowed me to perform research on some very exciting aspects of Hantavirus molecular biology.

Final Report

Date: January 8, 1997

Name: Yu Lin

Research laboratory: Dept. of Neuropharmacology, Div. of Neurosciences, WRAIR Building 40
Washington, D.C.

Date of Tenure: 07-17-94 to 01-17-98

Title of Research Proposal:

Mechanism and control of excitotoxic neuronal injury induced by brain trauma or hypoxia

Research Advisor: Joseph Long and Frank Tortella

Are you on leave from a professional post? No

Professional society office hold during tenure:

Society of Neuroscience
International Brain Research Society

Professional Travel during Tenure:

Domestic	International
Washington, D.C., 3 days	Beijing, China, 5 days

Seminars or Lectures Delivered at Universities and/or Institutes:

1. Presented at Div. of Neurosciences, WRAIR
   Title: ACPC protected against NMDA receptor mediated neuronal injury
2. Presented at NIH
   Title: Is elevated intracellular calcium essential for NMDA receptor-mediated neuronal injury?
3. Presented at Div. of Clinical Physiology, WRAIR
   Title: Is elevated intracellular calcium essential for NMDA receptor-mediated neuronal injury?
4. Presented at University of Uniformed Services
   Title: Is elevated intracellular calcium essential for NMDA receptor-mediated neuronal injury?

Summary of Research During Tenure:

During my associateship tenure, my studies have concentrated on determining the mechanisms of neuronal cell injury and repair and developing relevant therapeutic approaches. Quantitative assessment of "real time" cytoplasmic calcium mobilization in primary cell cultures and the relevance of changes in calcium concentration to cell injury have been carried out in my project by detection of a calcium sensitive fluorescent dye with a confocal microscope. The result challenges the theory that a sustained elevation of intracellular calcium in response to over-activation of excitatory amino acid receptors is essential/critical for the final excitotoxic neuronal degeneration. We have been led to reevaluate the roles of calcium and sodium

mobilization in cell injury.

Research in Progress:

1. What cytosolic components, if not the elevation of intracellular calcium concentration, play a critical role that bridges activation of cell membrane glutamate receptors and final cell injury?
2. Reevaluation of the roles of sodium mobilization and sodium channels in cell injury.
3. Potential therapeutic significance of compounds targeting at sodium channel to neuronal cell injury and repair.

Presentations at Scientific Meetings or Conferences:

1. Lin, Y. and J.B. Long, 1996. Is elevated intracellular calcium essential for NMDA receptor-mediated neuronal injury? J. Biomed. Sci. 3:137. The Third Annual Joint Scientific Symposium of NIH/FDA CAA and Washington, DC Chapter of SCBA
2. Lin, Y., and J.B. Long, 1996. 1-aminocyclopropanecarboxylic acid protects against NMDA receptor-mediated injury to cultured spinal cord neurons both acutely and through desensitization. Soc. for Neurosci. 22: 69.
3. Lin Y., et al, 1997. Differential effects of the sodium channel blockers mexiletine and QX-314 in primary rat cerebellar neurons: I. Protection against glutamate or hypoxic injury. Soc. Neurosci Abstr. 23: 1475.
4. Dave, JR., M.J. Koening, Y. Lin, et al., 1997. Differential effects of the sodium channel blockers mexiletine and QX-314 in primary rat cerebellar neurons: II. Glutamate or KCl-mediated calcium mobilization. Soc. Neurosci Abstr. 23: 1475.

Publications and Papers Resulting from NRC Associateship Research:

1. Lin, Y. and J.B. Long, 1996. Is elevated intracellular calcium essential for NMDA receptor-mediated neuronal injury? J. Biomed. Sci. 3:137.
2. Lin, Y., and J.B. Long, 1996. Acute or prolonged exposure to 1-aminocyclopropanecarboxylic acid protects spinal neurons against NMDA toxicity. European J. Pharmacol. 318:491-496.
3. Lin, Y., and J.B. Long, 1997. Prolonged preexposure to 1-aminocyclopropanecarboxylic acid protects against subsequent glutamate toxicity *in vitro*. European J. Pharmacol. 329: in press
4. Lin Y., et al, 1997. Differential effects of the sodium channel blockers mexiletine and QX-314 in primary rat cerebellar neurons: I. Protection against glutamate or hypoxic injury. Soc. Neurosci Abstr. 23: 1475.
5. Dave, JR., M.J. Koening, Y. Lin, et al., 1997. Differential effects of the sodium channel blockers mexiletine and QX-314 in primary rat cerebellar neurons: II. Glutamate or KCl-mediated calcium mobilization. Soc. Neurosci Abstr. 23: 1475.
6. Lin, Y., and J.B. Long, 1998. Prolonged preexposure to 1-aminocyclopropanecarboxylic acid differentially alters NMDA receptor agonist and antagonist potencies in spinal cord neuronal cultures. in preparation

Patent or Copyright Applications Resulting from NRC Associateship Research:

None

Future Position and Address and /or Forwarding Address:

Dr. Yu Lin
19352-101 Circle Gate Dr.
Germantown, MD 20874

Appraisal of the Associateship Programs:

The NRC Associateship program provides both opportunity and flexibility for young scientists in their career

development. The travel funds are generously sufficient and encourage scientific communication. I wish to thank my host institute, the Walter Reed Army Institute of Research and in particular, the Department of Neuropharmacology, for their support in the past 3.5 years. I wish to express my sincerest gratitude to my mentors, Dr. Joseph Long and Frank Tortella, and a special thanks to Dr. Wayne Jonas, the director of the Office of Alternative Medicine at NIH. It would have been impossible for me to fruitfully complete my tenure at NRC without their support.

9499970

NATIONAL RESEARCH COUNCIL
Resident Research Associateship Final Report

1) Date: April 14, 1998

2) Name: Dr. Barbara J. Meyer

3) Name of Laboratory and Location: USAMRIID
Ft. Detrick, MD

4) Dates of Tenure: 2/1/95 to 1/31/98

5) Title of Research Proposal:

Characterization of Genetic Properties of Hantaviruses During Acute and Persistent Infections

6) Name of Research Advisor: Dr. Connie Schmaljohn

7) Are you on leave from a professional post? no

8) Professional Society Offices Held During Tenure: none

9) Professional Travel During Tenure:

Attended 4 scientific conferences, as listed in 14 below.

10) Seminars or Lectures Delivered at Universities and/or Institutes: none

11) Summary of Research During Tenure:

This research has defined several details concerning the life cycle of hantaviruses during both acute and persistent infections in cultured cells and has determined that persistent infections of hantaviruses are not due to the common mechanisms used by most other persistent viruses, but that persistence is associated with small defects in one region of the genome. Based on these data, I have proposed a testable model of the molecular mechanism of hantavirus persistence. I previously proposed this model for another persistent virus, LCMV, during my work at the University of Minnesota before I started my NRC tenure at USAMRIID to examine persistent infections of hantaviruses.

12) Research in Progress:

We have been awarded a VA/DoD grant to study hantavirus persistence in a natural animal host and to test the molecular model I have proposed for persistence.

13) Publications Resulting from NRC Associateship research
a) Publications in peer-reviewed journals:

Schmaljohn, C., L. Vanderzanden, M. Bray, D. Custer, B. Meyer, D. Li, C. Rossi, D. Fuller, J. Haynes, and J. Huggins. 1997. Naked DNA vaccines expressing the prM and E genes of Russian spring summer encephalitis virus and central european encephalitis virus protect mice from homologous and heterologous challenge. *J. Virol.* 71: 9563-9569.

b) Manuscripts in preparation:

Meyer, B.J. and C. S. Schmaljohn. 1998. Molecular analysis of hantavirus persistent infections suggests that terminally deleted L RNAs have a causal role in persistence.

14) Presentations at Scientific Meetings:

a) Meyer, B.J. and C. S. Schmaljohn. 1996. Molecular characterization of hantavirus persistent infections. ASV 15th Annual Meeting, London, Ontario, Canada.

b) Meyer, B.J. and C. S. Schmaljohn. 1996. Molecular characterization of hantavirus persistent infections. ASV 15th Annual Meeting, London, Ontario, Canada.

c) Meyer, B.J. and C. S. Schmaljohn. 1997. Molecular analysis of hantavirus persistent infections suggests that terminally deleted L RNAs have a causal role in persistence. 10th International Conference on Negative Strand Viruses, Dublin, Ireland.

d) Meyer, B.J. and C. S. Schmaljohn. 1998. Molecular analysis of hantavirus RNA during persistent infections. The Fourth International Conference on HFRS and Hantaviruses, Atlanta, GA.

15) Patents, Copyrights, or Grant Awards Resulting from NRC Associateship Research:

Veterans Affairs and the Department of Defense Grant Award in Mechanisms of Emerging Pathogens. 1998 - 2000

16) Future Position and Address:

Microbiologist/Contractor

Dr. Barbara J. Meyer
Virology Division
USAMRIID
MCMR-UIV-M
1301 Ditto Avenue
Ft. Detrick, MD 21702-5023

9526800 BM/LH/ik

Morris Jim

To: Judy Nyquist PhD; Lisa Bevell
Subject: Final Report

20 August 98
Via email; Original via U.S. Mail

NRC Final Report

[1] Date:
20 August, 1998

[2] Name:
Jim Morris, PhD

[3] Name of Laboratory or Center and Location
The U.S. Army Medical Research Institute for Chemical Defense [USAMRICD], Edgewood, MD

[4] Dates of Tenure
11 Sept 95 to 10 Sept 98

[5] Title of Research Proposal
Effects of Pharmacological Treatment on Brain Neurotransmitters During Soman-Induced Seizures

[6] Name of Research Advisor
Tony Shih, PhD

[7] Are you on leave from a professional post?
No

[8] Professional Society Offices held during tenure?
None

[9] Professional Travel During Tenure. List locations and dates.
Society for Neuroscience Meetings, Washington DC, Nov 1996.

Experimental Biology '98 Meetings and IBC's New Developments in Glutamate Pharmacology - Therapeutic Implications Meeting, San Francisco, CA April, 1998

NIH meetings: The Glutamate Cascade: Common Pathways of Central Nervous System Disease States, Bethesda, MD May, 1998;

Medical Defense Bioscience Review, Hunt Valley, MD, May-June, 1998.

[10] Seminars or Lectures delivered at Universities &/or Institutes
"*In vivo* effects of biperiden and diazepam on brain cholinergic and aminergic neurotransmitters during soman-induced seizures"

Presented to Military Medical Scientists at USAMRICD, Edgewood, MD, April 1998.

[11] Summary of Research During Tenure [100 words or less; no symbols, etc]
I evaluated the effects of diazepam and biperiden on brain levels of acetylcholine, choline,

glutamate, and GABA during soman-induced seizure activity. The findings were that biperiden turned soman-induced seizures off faster than diazepam. In the presence of diazepam and biperiden, large decreases in soman-induced levels of acetylcholine occurred. However, in the presence of biperiden, a differential effect occurred with acetylcholine between seizure terminated and seizure not terminated animals. Seizure terminated animals had large decreases in acetylcholine, but acetylcholine was unchanged in animals whose seizures were not terminated by biperiden. With diazepam, there was no difference in acetylcholine between seizure terminated and seizure not terminated animals. Both drugs were similar in reversing the effects of soman on choline and glutamate, and but had no effect on GABA.

[12] Research in progress.

I have data collected for diazepam and biperiden effects on brain levels of aspartate, glutamine, glycine and taurine during soman-induced seizure activity.

Also, I have control data [basal, saline, diazepam, and biperiden] for acetylcholine, choline, aspartate, glutamate, glutamine, glycine, taurine, and GABA.

Other studies initiated on the project involve scopolamine and trihexyphenidyl effects on acetylcholine, choline, aspartate, glutamate, glutamine, glycine, taurine, and GABA.

[13] Publications and papers: complete citations, separate by journal, book chapter, manuscripts in preparation, etc.

Manuscript in Preparation:

Morris JL, McDonough, JH Jr., and Shih T-M. Diazepam and biperiden in vivo effects on brain cholinergic and aminergic systems during soman-induced seizure activity. Biochem. Pharmacol., in preparation, 1998.

[14] Presentations at Scientific Meetings or Conferences - give complete reference, as well as meeting name and location.

Presented Poster entitled:
"*In Vivo* Effects of Biperiden and Diazepam on Brain Cholinergic and Aminergic Neurotransmitters During Soman-induced Seizures", presented at:

Experimental Biology '98, San Francisco, CA; The FASEB Journal, Abstracts Part II, 12 (5):A750, April 1998;

IBC's New Developments in Glutamate Pharmacology - Therapeutic Implications, San Francisco, CA, April 1998;

NIH meeting: The Glutamate Cascade: Common Pathways of Central Nervous System Disease States, May, 1998;

Medical Defense Bioscience Review, Hunt Valley, MD, May/June, 1998.

[15] Patents or copywrights

None

[16] Future position and/or forwarding address [for tax statement]

I have yet to locate a future position. For now, please mail my tax statement to:

Jim Morris PhD
1410 Primrose Place
Belcamp, MD 21017

[17] Appraisal of the Associateship Programs [usefullness to you and suggestions]

Overall I felt the program at USAMRICD was very good. The equipment and facilities are very adequate. There was good opportunity for collaboration. I was able to make a significant

neuropharmacological contribution to the Army's mission of understanding of how soman-induced seizures are terminated and which drugs seem to work best. I was able to travel to meetings and discuss my findings. I have a manuscript in preparation. I appreciate very much the support and encouragement I received from Dr. Shih, Dr. McDonough, Dr. Hackley, the Institute [USAMRICD], and NRC.

FINAL REPORT

(1) DATE
28 April, 1998

(2) NAME AND ID NUMBER
Vicki L. Pierson
ID 951665

(3) NAME AND LOCATION OF LABORATORY OR CENTER
USAMRIID, Ft. Detrick, MD 21702

(4) DATES OF TENURE
6 September, 1995 to 19 January, 1998

(5) TITLE OF RESEARCH PROJECT
Regulation of the *Yersinia pestis* Capsular Antigen Operon by Caf1R, a Member of the AraC Family of Virulence Regulators

(6) RESEARCH ADVISOR'S NAME
Patricia L. Worsham, Ph.D.

(7) ARE YOU ON LEAVE FROM A PROFESSIONAL POST?
No.

(8) INTERNATIONAL POSTS HELD DURING TENURE
None

(9) PROGRAMMATIC TRAVEL DURING TENURE
96th General Meeting of the American Society for Microbiology
May 19-23, 1996, New Orleans, LA
97th General Meeting of the American Society for Microbiology
May 4-9, 1997, Miami, FL
1996-97 Quarterly meetings of the Maryland Branch of the American Society for Microbiology
Baltimore, MD

(10) SCIENTIFIC SEMINARS, MEETINGS, AND/OR CONSULTATIONS
Research seminar, USAMRIID Bacteriology Division, Nov. 16, 1995
Plague vaccine Review and Analysis, USAMRIID, Feb. 23, 1996
Research Review, USAMRIID Bacteriology Division, Dec. 18, 1997
Frederick County Public Schools, Intramural Research Lab Consultant (volunteer), ongoing

(11) SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES
National Cancer Institute Spring Research Festival, May 22, 1997
Maryland Branch of the American Society for Microbiology, May 23, 1997

(12) MEETINGS ATTENDED BY SPECIFIC INVITATION
See (11) above

(13) TEACHING, IF ANY, AS AN ASSOCIATE
UMAB School of Medical Technology Laboratory Intern Training, May-August 1996
Frederick County Public Schools, Advanced Placement Biology Bacterial Genetics Labs, 1997

(14) WORK IN PROGRESS
I have arranged to continue my present work through collaborations with Dr. Patricia Worsham at USAMRIID and Dr. James Sawitzke at the National Cancer Institute. Dr. Worsham and I are

investigating the underlying defect(s) contributing to the capsule-negative phenotype of spontaneous mutants of Y. pestis isolated from experimentally infected animals. Dr. Sawitzke and I are studying the role of small DNA binding proteins in bacterial chromosomal condensation. I anticipate that both of these collaborations will result in publication within this calendar year.

(15) SUMMARY OF RESEARCH DURING TENURE

We discovered in the DNA flanking the capsular operon of *Yersinia pestis* an insertion sequence, which may influence operon regulation and suggests the presence of a pathogenicity island. We isolated spontaneous capsule negative mutants that could not be complemented in trans with the intact operon, and continue studying the nature of their defect(s). Mutants that could be complemented produced less capsule than the parent strain, despite a vastly higher copy number. We are investigating the role of DNA topology in this process. A histone-like protein from *Y. pestis*, which modulates the expression of virulence factors in *Y. enterocolitica*, partially complements defective chromosome compaction in *E. coli*.

(16) PUBLICATIONS AND PAPERS RESULTING FROM RESEARCH AS AN ASSOCIATE

Development of *E. coli* host strains tolerating unstable DNA sequences on high copy ColE1 vectors, by Vicki L. Pierson and Gerard J. Barcak, submitted to *FOCUS*, April 1998; also see (14) above.

(17) PATENTS APPLIED FOR AS A RESULT OF RESEARCH AS AN ASSOCIATE

None

(18) FUTURE POSITION AND ADDRESS OR CURRENT FORWARDING ADDRESS

Vicki L. Pierson, Ph.D.
Joint Vaccine Acquisition Program
1436 Porter St.
Ft. Detrick, MD 21702

(19) APPRAISAL OF THE ASSOCIATESHIP PROGRAMS

This fellowship was an invaluable aid in my professional development, and was directly responsible for my being competitive for the position I now hold. USAMRIID has no opportunities for postdoctoral training other than through the National Research Council, and the experience I gained here is not available anywhere else in this country. I am deeply indebted to the Associateship Program, and recommend it highly to anyone considering tenure with the NRC.

Joint Vaccine Acquisition Program
1436 Porter St.
Ft. Detrick, MD 21702

Judith K. Nyquist, Ph.D.
Deputy Director and Program Administrator
Associateship Programs
Office of Scientific and Engineering Personnel
National Research Council
2101 Constitution Ave.
Washington, DC 20418

Dear Dr. Nyquist,

I would like to express my appreciation for the Research Fellowship that I held at USAMRIID through your program. The fellowship gave me the opportunity to refine my research skills and acquire new ones. The experience and professional growth I experienced during my tenure was directly responsible for my obtaining the position I now hold as Technical Product Manager at the Joint Vaccine Acquisition Program here at Ft. Detrick, Maryland. Due to the unique relationship between JVAP and USAMRIID, I am able to retain a collaborative interest in the research I initiated there. Thus it seems that I have the best of both worlds, and I feel that I used my fellowship to its best advantage. Attached please find my final report; I apologize for not being more prompt, but the transition from the bench to the office has taken longer than I had anticipated. I hope this has not inconvenienced you. Again, thank you for the opportunity to develop my professional career through your program.

Sincerely,

Vicki Pierson

Vicki L. Pierson, Ph.D.

9211760

National Research Council
Associateship Programs
2101 Constitution Avenue, NW, TJ 2114
Washington, DC 20418

Final Report

November 19, 1997

Peter Pushko, Ph. D.
Virology Division
US Army Medical Research Institute for Infectious Diseases
Fort Detrick, Frederick, MD 21701

Tenure: May 20, 1994 to November 19, 1997

Title of research proposal: "Development of a Venezuelan Equine Encephalitis (VEE) virus replicon as a universal vaccine vector"

Research Adviser: Jonathan F. Smith, Ph. D.

Professional travel during tenure:

1996: Meeting "Molecular approaches to the control of infectious diseases". Cold Spring Harbor, New York.
1995: 44th Annual Meeting of the American Society of Tropical Medicine and Hygiene. San-Antonio, Texas

1996: Xth International Congress of Virology. Jerusalem, Israel
1995: IVth International Symposium on positive strand RNA Viruses. Utrecht, The Netherlands.

Summary of research during tenure

The universal vaccine vector was developed from the attenuated Venezuelan equine encephalitis virus (VEE) replicon. Diring tenure, this vector was improved to introduce safety features allowing veterinary and human use. These features included i) separation of the VEE helper RNA into two independent RNA species, ii) optimization of the helper RNA structure for maximal replicon encapsidation

and minimal regeneration of replicating VEE, and iii) mutagenesis of the VEE capsid autoprotease active site. The safety, immunogenicity, and protection capacity of the VEE vaccine vectors have been demonstrated in a variety of animal models using genes from influenza, Lassa, Rift Valley Fever, and Ebola viruses.

Research in progress

Evaluation of the safety and efficacy of the VEE replicon vaccine vector in primates.

Presentations at scientific meetings or conferences

1. Pushko, P., Parker, M., Ludwig, G. V., Davis, N. L., Johnston, R. E., and Smith, J. F. (1996). Development of a generic vaccine delivery system based on a Venezuelan equine encephalitis (VEE) virus replicon. 45th Annual Meeting of the American Society of Tropical Medicine and Hygiene. Baltimore, Maryland
2. Pushko, P., Parker, M., Ludwig, G. V., Davis, N. L., Johnston, R. E., and Smith, J. F. (1996). A generic vaccine vector based on a Venezuelan equine encephalitis virus (VEE) replicon. Meeting "Molecular approaches to the control of infectious diseases". Cold Spring Harbor, New York.
3. Pushko, P., Parker, M., Ludwig, G. V., Davis, N. L., Johnston, R. E., and Smith, J. F. (1996). A nonreplicative system from attenuated Venezuelan equine encephalitis virus (VEE) as a novel vaccine delivery vector. Xth International Congress of Virology. Jerusalem, Israel.
4. Pushko, P., Parker, M., Ludwig, G. V., Davis, N. L., Johnston, R. E., and Smith, J. F. (1995). Development of RNA replicon and helper systems from attenuated strains of Venezuelan equine encephalitis (VEE) virus. 44th Annual Meeting of the American Society of Tropical Medicine and Hygiene. San-Antonio, Texas
5. Pushko, P., Parker, M., Ludwig, G., Davis, N., Johnston, R., Smith. (1995). Replicon and helper systems generated from vaccine strains of Venezuelan Equine Encephalitis (VEE) virus for expression and vaccine delivery. IVth International Symposium on positive strand RNA Viruses. Utrecht, The Netherlands.

Publications and papers resulting from NRC associateship research

Publications

Pushko, P., Parker, M., Ludwig, G. V., Davis, N. L., Johnston, R. E., and Smith, J. F. (1997). Replicon-helper systems from attenuated Venezuelan equine encephalitis virus: expression of heterologous genes in vitro and immunization against heterologous pathogens in vivo. *Virology*. In press.

Book chapters:

Pushko, P., Parker, M., Ludwig, G., Geisbert, J., Negley, D., Schmaljohn, A, Jahrling, P. B., Sanchez, A., and Smith, J. F. (1997). Venezuelan equine encephalitis virus (VEE) replicon vector: immunogenicity studies with Ebola NP and GP genes in guinea pigs. *In:* Vaccines97, p. p. 43-83. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA.

Patents submitted

Johnston, R. E., Davis, N. L., Smith, J. F., Pushko, P., Parker, M., Ludwig, G. (1995). Alphavirus replicon systems.

Dr. J. Nyquist
National Research Council
Associateship Programs
2101 Constitution Avenue, NW, TJ 2114
Washington, DC 20418

November 19, 1997

Dear Dr. Nyquist:

Enclosed please find my final report. Please let me know if you need any additional information. After the completion of my NRC Associateship, I hope to stay at the US Army Medical Research Institute, Fort Detrick, Frederick.

I thank you and all NRC personnel for continuous support during my NRC Associateship. It has been a great pleasure to participate in the NRC Associateship program. Thanks,

Yours sincerely,

Peter Pushko

Peter Pushko, Ph.D.
Virology Division, USAMRIID
Fort Detrick, Frederick, MD 21701
Phone (301) 619-4949
Fax (301) 619-2290

9608060

FINAL REPORT

1. Date: February 4, 1998

2. Name: Shanker P. Reddy, Ph.D.

3. Name of the laboratory: Bacteriology, USAMRIID, 1425 Porter street, Fort Detrick, Frederick, MD 21702-5011.

4. Dates of the tenure: March 3, 1997 to March 2, 1998.

5. Title of the research proposal: Analysis of the effects of the V antigen and plasminogen activator of *Yersinia pestis* on the host response to infection.

6. Name of research advisor: Dr. Susan L. Welkos

7. Are you on leave from a professional post: No

8. Professional society offices held during tenure: None

9. Professional travel during tenure: ASM meeting in Miami, FL. May 4-8, 1997

10. Seminars/lectures delivered at universities and/or institutes: Department of Microbiology and Cell Science, University of Florida, Gainesville, FL. October 24, 1997.

11. Summary of Research during tenure: The project is on the mechanism of immunosuppression by *Yersinia pestis* by V antigen: V antigen is a regulatory protein required for calcium dependent growth, expression of virulence genes encoded in the Lcr plasmid and possibly involved in the inhibition of synthesis of preinflammatory cytokines by neutophils as a mechanism of suppression of the early host immune response to infection. To investigate the latter role, in-vitro assays were set up with the isolated human neutrophils, treated previously with V antigen or with buffer alone, followed by activation using PMA or FMLP or TNF-alpha. The activation was monitored by the release of superoxide radical or hydrolytic enzymes such as beta glucuronidase and beta glucosaminidase. Even after several repeats of these experiments with varying parameters, V antigen did not inactivate neutrophils. In other words, the control neutrophil activation values were similar to the values obtained in the presence of V.

12. Research in progress: 1) For immunosuppression of neutrophils, besides V antigen, other virulence factors, such as Yops, would be needed. This is

11. Summary of research on a **second** project on the involvement of bacterial immunophilins in the host immunosuppression by *Yersinia*:

Bacterial immunophilins are peptidyl prolyl isomerases (pplases) that catalyze cis-trans transformation of prolyl residues in proteins and are shown to be required for the virulence of*Legionella, Chlamydia* and mycoplasmas. Also intriguing is the inhibition of protein kinase C (PKC) by the pplase from *Legionella*, which suggests that pplases may be involved in immunosuppression. There are two classes of pplases that bind immunosuppressive drugs such as cyclosporine A and FK 506 and are referred to as cyclophilins or FK506 binding proteins (FKBPs), respectively.

As the first step, the genes coding for pplases (both cyclophilins and FKBPs) from *Y.pestis*, *Y.enterocolitica* and *Y.pseudotuberculosis* were cloned by PCR. Several forward and reverse primers were constructed using the sequences from the N and C-terminii, respectively, of the homologous regions of cyclophilins and FKBPs from other prokaryotes. Annealing temperatures and Mn^{2+} concentrations were manipulated to obtain unique PCR products. The sizes of the PCR products for cyclophilins from the three *Yersinia* spp. ranged from 250 to 280bp, and for FKBPs, the sizes ranged between 780bp and 850bp. The PCR fragments were gel purified and cloned into pNoTA. These PCR cloned immunophilin genes are being sequenced.

12. Research in progress: The DNA sequences from the PCR cloned cyclophilins and FKBPs from the three *Yersinia* spp. should be compared for homologies with immunophilins from other sources and the DNA fragments of interest will be used as probes for isolating larger DNA fragments from the respective genomic DNA digests of the *Yersiniae* by Southern and Colony hybridizations. These larger fragments will then be recloned into a suicide vector, pCVD442, and used in the site-directed mutagenesis of the *Yersiniae*. The mutants will be further characterized for the presence of virulence factors (V and Yops) by immunoblots and subjected to studies on invasion and virulence using both in-vitro and in-vivo mouse models.

easy to demonstrate by using combinations of purified V and Yops in the same neutrophil activation assays described above. Antibodies against V and Yops are available now for further testing. 2) identification of interacting neutrophil receptor by using the fluorescent labeled V antigen 3) identification of involvement of immune cells other than neutrophils

13. Publication and papers resulting from NRC associateship: None at this time. One publication might result from the immunophilins work later.

14. Presentations at scientific meeting or conferences: None

15. Patent or copyright applications from NRC associateship research: the sequences of immunophilins from *Yersinia* spp. could be patented.

16. Futute position and forwarding address: Project Director, Megan Health, Inc., 3655 Vista Ave, St. Louis, MO 63110.

17. Appraisal of the associateship programs: I have gained knowledge on the immunopathogenesis by pathogenic bacteria and vaccinology.

Shanker P. Reddy, Ph.D.
Bacteriology Division, USAMRIID
1425 Porter Street, Fort Detrick
Frederick, MD 21702-5011

Voice Mail: 301-619-4918 FAX: 301-619-2152
E-mail: shanker_reddy@ftdetrck-ccmail.army.mil

February 4, 1998.

Dear Dr. Nyquist

I am planning to complete my NRC Senior associateship as of March 2, 1998 and I do not plan on renewing it. I have enclosed the Final Report and the forwarding address. I do not want the medical insurance coverage and the Worker's Compensation for the month of March.

Thank you for this opportunity.

Sincerely,

Shanker Reddy

94990S0

FINAL REPORT

1) DATE: March 23, 1998

2) NAME (AND NRC ASSOCIATESHIP ID. NUMBER IF KNOWN):
M. KAMAL UDDIN SAIKH

3) NAME OF LABORATORY OR CENTER AND LOCATION:
USAMRIID
Dept. of Immunology and Molecular Biology
1425 Porter street
Fort Detrick
Frederick, MD 21702

4) DATES OF TENURE:
April 3, 1995 to April 2, 1998.

5) TITLE OF RESEARCH PROPOSAL:
Role of dendritic cells in delivering antigen in polynucleotide based vaccination and in MHC class II salvage pathway antigen presentation.

6) NAME OF RESEARCH ADVISER:
DR. Robert G. Ulrich

7) ARE YOU ON LEAVE FROM A PROFESSIONAL POST?
If so, list position or title and address.
No.

8) PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE
Member of International Society of Vaccine and American Association for the Advancement of Science

9) PROFESSIONAL TRAVEL DURING TENURE
List location(s) and date(s) of travel to scientific meetings. List foreign meetings separately

1) San Francisco, CA ; July 23 - 29, 1995; The 9th International

Congress of Immunology.
2) Hilton Head, South Carolina; March 20-26, 1996; Keystone Symposia on Lymphocyte Activation

3) New Orleans, LA ; June 2-6, 1996; FASEB meetings

4). Leesburg, Virginia; Sept. 8-12, 1997; International Society for Vaccines Meeting

5) Santa Fe, New Mexico; March 7-13, 1998; Keystone Symposia on Cellular and Molecular Biology of Dendritic cells.

10) SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES

List location(s) and date(s).

Indian Institute of Chemical Biology, Calcutta, INDIA; August 7, 1997.

Two RIID Seminars at USAMRIID, April, 1996 and April 1997; One Research Festival Seminars at Ft. Detrick/NCI, May 1997.

11) SUMMARY OF RESEARCH DURING TENURE

List significant findings in concise form (100 words or less).

Please do NOT use Greek letters or mathematical signs and symbols.

The role of dendritic cells in supporting the induction of antibody and cytotoxic T lymphocyte responses to a plasmid based DNA vaccine encoding the C fragment polypeptide of tetanus toxin was examined. . Dendritic cells collected from the spleens of mice that were injected with plasmid into muscle sites transferred toxin-specific immunity to nonimmune mice. These results confirmed that dendritic cell present antigen presentation in primary immune responses to plasmid-based DNA vaccines. The interrelationship between cell maturation and the acquisition by human DCs of the ability to process and present antigens was also studied. A unique CD14-, CD34- progenitor cells, lacking cell-surface HLA-DR, differentiated into DCs which acquired the ability to process and present complex polypeptides from tetanus toxin that coincided with the transcriptional activation of HLA-DR by the class II transactivator CIITA, the intracellular biosynthesis of MHC class II molecules. A reduction of cytoplasmic MHC class II molecules and an increase in antigen-specific T-cell stimulatory responses were detected

after a lag of 3 days in culture. Surface expression of HLA-DR declined dramatically by 6 days in culture. The loss of antigen processing appeared to be the result of TNF-alpha-dependent apoptosis.

12) RESEARCH IN PROGRESS

To improve the immunogenicity of DNA vaccines especially for inducing strong antibody response at the levels of protein immunization, studies are underway to shift the processing pathway for class II restricted antigen recognition towards Th2 type responses. My interest in human dendritic cell research is currently focused on MHC class II linked functional maturation and the regulation of activation induced cell death in these cells.

13) PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH.

Provide complete citation(s), including author(s), full name of journal, volume number, page number(s), and year of publication. Please list separately:

(a) Publications in peer-reviewed journals;

(b) Books or book chapters; and

(c) Manuscripts in preparation, manuscripts submitted.

(a).Publications:

1). Saikh, K.U., Brandler, P., Sesno, J., and Ulrich, R.G. (1997). Are DNA-based vaccines useful for protection against secreted bacterial toxins? Tetanus toxin as a test case. **(in press, Vaccine).**

(c) Manuscripts submitted

1). Saikh, K.U., Conlon., K., and Ulrich, R.G. (1998). Antigen presentation by dendritic cells is controlled by a burst of HLA-DR expression and is prolonged by TNF α from activated T cells **(submitted).**

2). Brandler, P., Saikh, K.U., Heath, D., Friedlander, A and Ulrich, R.G. (1998). Weak anamnestic responses of inbred mice to Yersinia F1 genetic vaccine are overcome by boosting with F1 polypeptide while outbred mice remain nonresponsive **(submitted)**.

(c) Manuscripts in preparation

1). Saikh, K.U., Brandler, P., Krakauer, and Ulrich, R.G. (1998). Immune response to priming antigens remain plastic while memory

responses are preferentially dictated by the antigen presentation pathway **(In preparation)**

2) Ulrich, R. G., Saikh, K. U. et al. (1997). Analysis of the human T cell response to botulinum and tetanus toxin C fragment epitopes **(In preparation).**

14) PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES

Provide complete reference with authors, title, abstract or proceeding citation, and meeting name and location for international and domestic.

Presentation In Professional Meetings:

1. Saikh, K. U. and Ulrich, R. G. (1995). A simple method for the isolation and characterization of enriched populations of dendritic cells from mouse spleen. Abstr. #04609, The 9th International Congress of Immunology, July 23 - 29, San Francisco.

2. Saikh, K. U., and Ulrich, R. G. (1996). Dendritic cells transfected with plasmid coded antigen efficiently transfer antibody response to naive recipient mice. Abstr.#2067 ;Keystone Symposia on Lymphocyte Activation, March 20-26, South Carolina.

3. Saikh, K. U., Conlon, K., and Ulrich , R. G. (1996). Functional maturation of human dendritic cells precedes through a MHC class II negative precursor. Abstr.#1238 ; FASEB meetings. June 2-6, New Orleans;

4. Ulrich, R. G., Brandler, P., Dyas, B., Sesno, J., and Saikh, K.U. (1996). Comparison of exogenous and plasmid-mediated routes of vaccination for MHC class II-dependent antigens. Abstr.#2726 ; FASEB meetings, June 2-6, New Orleans.

5. Brandler, P., Saikh, K.U., Heath, D, Friedlander, A and Ulrich, R.G. (1997). *Yersinia pestis* F1 genetic vaccine in combination with recombinant F1 protein optimally promotes both cellular and antibody mediated immunity. Abstr. presented at the International Society for Vaccines Meeting, Sept. 8-12, Leesburg, Virginia.

6. Saikh, K.U., Conlon., K., and Ulrich, R.G. (1998). A unique dendritic cell from human peripheral blood exhibits a rapid increase and decrease in antigen processing that coincides with TNFα -linked apoptosis. Abstr.

#142, Keystone Symposia, Santa Fe, March 7-13, 1998.

15) PATENT OR COPYRIGHT APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RESEARCH

None

16) FUTURE POSITION AND ADDRESS AND/OR FORWARDING ADDRESS

(clearly indicate to which address you wish your tax statement mailed)

Investigator, Clinical Research Management, Inc.

Address to send tax statement :
8609 Watershed Court
Gaithersburg, MD 20877

17) APPRAISAL OF THE ASSOCIATESHIP PROGRAMS

Comment on the usefulness of the Associateships Program to you, and include suggestions for improvements.

The associateship awarded to me was very helpful in pursuing my research interest within the infra-structure and mission of USAMRIID. The laboratory and technical facilities including intellectual support and scientific interactions with Dr. Robert Ulrich strengthen my career on the understanding of antigen processing and presentation in the context of dendritic cell immunobiology in the field of vaccine deveopment. I gratefully acknowledge the NRC for offering me the associateship to continue my research. For the last three year I was fortunate and productive to generate results for publications.

9545590

Date: 12 June 1998

Name: Kausalya Santhanam

Laboratory: Walter Reed Army Institute of Research,
Department of Respiratory Research,
Division of Medicine, Bldg. 40,
Washington DC 20307.

Dates of Tenure: 5 July 1995 to 5 July 1998.

Title of Research Proposal: Neutrophil-mediated tissue injury: Role of oxidative stress and apoptosis.

Name of Research Adviser: Jayasree Nath

Are you on leave from a professional post: NO

Professional Society Offices held during Tenure: None

Professional Travel during the tenure:

1. Experimental Biology '96, April 14-17, 1996, Washington DC.

2. Phagocyte Workshop, May 3, 1996, Washington DC.

3. VI International Congress on Cell Biology, December 7-11, 1996, San Francisco.

4. Experimental Biology '97, April 6-9, New Orleans.

5. Phagocyte Workshop, May 2, 1997, Washington DC.

6. Key Stone Symposium on 'Endothelium', March 22-28, 1998, Lake Tahoe, Nevada.

7. Experimental Biology '98, April 18-22, 1998, San Francisco.

Seminars or Lectures delivered at Universities/Institutes: None

Summary of Research during Tenure:

Role of nitric oxide (NO) and reactive oxygen intermediates (ROI) in neutrophil-endothelial cell interactions leading to endothelial cell injury was studied. Adhesion and cytotoxicity of endothelial cells were augmented by addition of cytokines. An inverse correlation between NO and ROI was found to be the critical factor for maintaining the inflammatory responses in vivo. The results were further strengthened by using

neutrophils from chronic granulomatous disease patients which are defective in generating ROI.
Role of IL-12 in neutrophil-mediated bronchial epithelial cell killing was studied. It was found that IL-12 increases adhesion and cytotoxicity probably by releasing IL-8. Increased IL-8 levels were detected in IL-12 primed epithelial cells and IL-8 stimulation of epithelial cells resulted in increased expression of cell surface adhesion molecules.

Research in Progress:

Toxic gas exposure, either ambient or supra ambient, has been proven to be hazardous to humans. A novel cost effective technique for exposing cells to nitrogen dioxide has been adapted in the laboratory in order to define the early biomarkers of injury. The cells used are bronchial epithelial cells and human umbilical vein endothelial cells. Preliminary results show a decrease in the proliferative rate of nitrogen dioxide exposed cells as against the unexposed cells. Assays to detect early permeability changes and morphological changes are being currently studied.

Publications and Papers resulting from NRC Associateship Research:

1. Interactive role of superoxide and nitric oxide in neutrophil-endothelial cell interactions. **Journal of Leukocyte Biology**, 1998, In Press.

Manuscript submitted:

1. Functional Significance of nitric oxide generation in neutrophils from normal and chronic granulomatous disease patients, **Journal of Leukocyte Biology**, 1998, manuscript submitted.

Manuscript in preparation:

1. Role of interleukin-12 on neutrophil-bronchial epithelial cell interactions. Manuscript in preparation.

2. Effect of nitrogen dioxide exposure to human endothelial and epithelial cells. Manuscript in preparation.

Presentations at Scientific Meetings or Conferences:

1. Neutrophil-Endothelial cell interactions: Role of Nitric Oxide. S. Kausalya, J. Nath, 1996. **The FASEB Journal**, A280. Experimental Biology '96, April 14-17, 1996, Washington DC.

2. Nitric Oxide generation in activated human neutrophils: Studies in unprimed and LPS-primed cells. J. Nath, P. Tulsi, K. Kray, S. Kausalya, 1996. **Journal of Investigative Medicine**, 44(3): 267A. Biomedicine '96, May 3-6, Washington DC.

3. Role of Nitric Oxide in neutrophil-mediated endothelial cell injury. S. Kausalya, J. Nath, 1996. **Molecular Biology of Cell**, 7: 658a. VI International Congress on Cell Biology, December 7-11, 1996, San Francisco.

4. Interactive role of Nitric Oxide and Reactive Oxygen Intermediates in neutrophil-endothelial cell interaction. S. Kausalya, J. Nath, 1997. **The FASEB Journal**, 11(3): A377. Experimental Biology '97, April 6-9, New Orleans.

5. Inverse Correlation between Superoxide and Nitric Oxide generation in activated human neutrophils: Studies in unprimed and endotoxin-primed cells. J. Nath, S. Kausalya, 1997. **The FASEB Journal**, 11(3): A644. Experimental Biology '97, April 6-9, New Orleans.

6. Role of Superoxide and Nitric Oxide in neutrophil-mediated endothelial injury: Studies with normal and chronic granulomatous disease neutrophils. J. Nath, S. Kausalya, 1997. **Journal of Investigative Medicine**, 45(3): 270A. Biomedicine '97, April 25-27, Washington DC.

7. Neutrophil-endothelial cell interactions: Inverse correlation between nitric oxide and superoxide anions. S. Kausalya, J. Nath, 1998, **Proceedings of the KeyStone Symposium on Endothelium**, p.90. KeyStone Symposium 1998, March 22-28, Lake Tahoe, Nevada.

8. Neutrophil-Bronchial Epithelial cell interactions: Role of IL-12 in Adhesion and Cytotoxicity. S. Kausalya, J. Nath, 1998. **The FASEB Journal**, 12(4): A645. Experimental Biology '98, April 18-22, San Francisco.

Patent resulting from NRC Associateship Research: None

Forwarding Address:
Kausalya Santhanam,
C/o K V Santhanam,
Flat # 116, Block 13, Jeevan Mitra Colony,
II Phase, J P Nagar,
Bangalore, 560078, INDIA.

Appraisal of the Associateship Programs:

The NRC Associateship program gives an opportunity to focus on a particular project to achieve meaningful results. The program also provides opportunities for fellows to attend scientific meetings and interact with other scientists.

Technion - Israel Institute of Technology
Faculty of Mechanical Engineering

Professor Avraham Shitzer
The James H. (Jimmy) Belfer Chair
in Mechanical Engineering

הטכניון — מכון טכנולוגי לישראל
הפקולטה להנדסת מכונות

פרופ' אברהם שיצר
הקתדרה להנדסת מכונות
ע"ש ג'ימס ה. בלפר

Dr. Avraham Shitzer
Department of Mechanical Engineering
Technion, Israel Institute of Technology
Haifa, Israel 32000

National Research council
Associateship Programs
2101 Constitution Ave., NW TJ 2114
Washington DC 80418
U.S.A.

Final Report

1) Date: Oct. 26, 1997
2) Laboratory: US Army Institute of Environmental Medicine, Natick, MA.
4) Dates of tenure: Aug. 12, 1996 - Sept. 29, 1997
5) Tittle of research proposal:
   Development of mathematical algorithms for simultaneous finger-tip temperatures and blood perfusion rates during cold stress.
6) Research adviser: Dr. Richard R. Gonzalez
7) On leave from the Technion, Israel Institute of Technology, Haifa, ISRAEL 32000, James H. Belfer, Professor of Mechanical Engineering.
8) Professional society offices held: none
9) Professional travel:
   (a) National Scientific meetings:
      1) International Mechanical Engineering Congress, Atlanta, GA. 19 - 21.11.96
      2) Experimental Biology '97, New Orleans, LA. 7 - 9.4.97
      3) Future of Biotechnology, Allerton, IL 18 - 21.4.97

   (b) Foreign Scientific meetings:
      1) The 7th International conference on Environmental Ergonomics, Jerusalem, Israel. 27.10 - 1.11.96

2) IDF - US Army Conference on Military Medicine, Jerusalem, Israel, 4 - 5.11.96

10) Seminars:
   (a) Michigan State University, East Lansing, MI. 17.4.97
   (b) University of Minnesota, Minneapolis, MN. 27.6.97

11) Summary of research:
   (a) Characterization of 3-phase response of cold-stressed fingers.
   (b) Energy economy and heat loss efficiency of cold-stressed fingers.
   (c) Modeling the thermal response of cold-stressed fingers.

12) Research in progress:
   (a) Short and long term adaptation of fingers to cold exposures.
   (b) Application of spectrum analysis techniques to study responses of cold-stressed fingers.

13) Presentations at scientific meetings:
   (a) A. Shitzer, Models to describe finger behavior under cold-stress. The 7th International Conference on Environmental Ergonomics, Jerusalem, Israel, 11/1996
   (b) A. Shitzer, Estimating endurance times by the behavior of cold-stressed fingers. IDF - US Army conference on Military Ergonomics Jerusalem, Israel 11/1996
   (c) Y. Chayut and A. Shitzer, Simulating the effects of a large blood vessel on the temperature field around a surface cryoprobe. ASME International Mechanical Engineering Congress and Exhibition, Atlanta, GA 11/1996.

   (d) A. Shitzer, S. Bellomo, L.A. Stroschein, R.R. Gonzales and K.B. Pandolf, Simulation of a cold-stressed finger including the effects of wind, gloves and cold-induced vasodilatation, Experimental Biology '97, New Orleans, LA 4/1997. (Abstract in: FASEB Journal, 11(3): A 289, 1997)

(e) A. Shitzer, Estimating endurance times by modeling the biothermal behavior of cold-stressed fingers. Allerton Workshop on the Future of Biothermal Engineering Allerton, IL 4/1997.

14) Publications:

(a) Peer-reviewed journals:

(1) A. Shitzer, L.A. Stroschein, M.W. Sharp, R.R. Gonzalez and K.B. Pandolf, Simultaneous measurements of finger-tip temperatures and blood perfusion rates in a cold environment. Journal of Thermal Biology, 1997 (in press).

(2) A. Shitzer, S. Bellomo, L.A. Stroschein, R.R. Gonzalez and K.B. Pandolf, Simulation of a cold-stressed finger including the effects of wind, glove and cold-induced vasodilatation. ASME Journal of Biomechanical Engineering, 1998 (in press).

(b) Book Chapter:

A. Shitzer, S. Bellomo, L.A. Stroschein, R.R. Gonzalez and K.B. Pandolf, Numerical model of the thermal behavior of an extremity in a cold environment including counter-current heat exchange between the blood vessels (tentative title). Solicited by Gordon and Breach International Series in "Engineering, Technology and Applied Science" volume on "Computer Techniques in Medical and Biotechnology Systems." 1998/9

(c) Manuscripts submitted:

(1) A. Shitzer, T.L. Endrusick, L.A. Stroschein, R.F. Wallace and R.R. Gonzalez, Characterization of a three-phase response in cold-stressed fingers. Submitted to the European Journal of Applied Physiology and Occupational Physiology.

(2) A. Shitzer, T.L. Endrusick, L.A. Stroschein, R.F. Wallace and R.R. Gonzalez, Heat loss efficiency economy of fingers during cold induced vasodilatation. Submitted to the Journal of Applied Physiology.

(15) Patents etc. - none

(16) Future position: James H. Belfer Professor of Mechanical Engineering, Faculty of Mechanical Engineering, Technion, Israel Institute of Technology, Haifa, Israel 32000

(17) Appraisal:

This is definitely one of the most useful and well managed research collaboration programs. It provides for viable and meaningful opportunities for interdisciplinary efforts and intellectual development of the associate while beneficially reflecting on the activity of the host laboratory. All involved at the NRC should be congratulated and encouraged to continue their good work !

9458251

FINAL REPORT

1) **DATE** December 25, 1998

2) **NAME** Leonard P. Wasieloski Jr.

3) **NAME OF LABORATORY OR CENTER AND LOCATION**

AMRIID, Ft. Detrick, Maryland.

4) **DATES OF TENURE**

April 24, 1995 to April 14, 1998

5) **TITLE OF RESEARCH PROPOSAL**

Expression of Nonreplicating Virus-like Particles of Viruses Causing Acute Hemorrhagic Fever

6) **NAME OF RESEARCH ADVISER** Dr. Kevin Anderson

7) **ARE YOU ON LEAVE FROM A PROFESSIONAL POST?** NO

8) **PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE** None

9) **PROFESSIONAL TRAVEL DURING TENURE**

Domestic Meetings:

July 8 to July 12, 1995. American Society for Virology annual meeting, Austin TX.

November 17 to November 21, 1995. American Society for Tropical Medicine and Hygiene annual meeting, San Antonio TX.

Foreign Meetings:

July 13 to July 17, 1996. American Society for Virology annual meeting, London Ontario.

September 21 to September 26, 1997. Tenth International Conference on Negative strand Viruses. Dublin, Ireland.

10) **SEMINAR OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES.** None.

11) **SUMMARY OF RESEARCH DURING TENURE.**

I constructed DNA plasmid vectors and recombinant viruses expressing NP, VP35, VP40, GP and VP24 Ebola virus genes for the purpose of developing reagents to analyze the expression Ebola virus-like particles. Proteins expressed from these gene products were found to be antigenically functional, and used to identify the specificity of a panel of monoclonal antibodies.

I developed a system for expressing RNA transcripts having authentic Ebola virus genomic

5' and 3' ends. These artificial genomes were transcribed from plasmids within cells, replicated by Ebola virus, encapsidated, assembled into particles. These Ebola virus-like particles have been passaged six times.

12) RESEARCH IN PROGRESS

I am in the process of developing high titer stocks of Ebola virus-like particles for experiments using immunoelectron microscopy and the monoclonal antibodies described above to identify the Ebola structural proteins associated with the particles. The synthetic RNAs are also being manipulated to identify the minimum sequence requirements for encapsidation, assembly and budding.

In addition, my former lab is engaged in collaborations with two other researchers at USAMRIID to use these mini-genomes for *in vitro* and *in vivo* Ebola replication assays. These assays, since they will not require infectious virus, will be a major advance for the safe study of Ebola virus replication

13) PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH

Gilligan K. J. , K. Anderson, J. B. Geisbert, L. P. Wasieloski, Jr., and P. B. Jahrling. Ebola viral proteins that elicit or inhibit protective immunity in guinea pigs. Manuscript in preparation.

Wasieloski, L.P. Jr., H. Alterson, K. Gilligan, and K. Anderson. Encapsidation, Assembly and budding of Ebola virus synthetic genomes. Manuscript in preparation.

14) PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES.

Wasieloski, L. P. Jr., K. J. Gilligan, B. Connolly, P. B. Jahrling, and K. Anderson. 1996. Antigenic and strain specificity of mouse monoclonal antibodies against Ebola Zaire virus. 15th annual meeting of the American Society for Virology.\

Anderson, K., K. J. Gilligan, J. B. Geisbert, L. P. Wasieloski, Jr., and P. B. Jahrling. 1997. Ebola viral proteins that elicit or inhibit protective immunity in guinea pigs. 10th International Conference on Negative Strand Viruses.

15) PATENT OR COPYRIGHT APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RESEARCH. None.

16) FUTURE POSITION AND ADDRESS AND/OR FORWARDING ADDRESS

Current Position:

Dr. Leonard P. Wasieloski
Diagnostic Systems Division
USAMRIID-Fort Detrick
1425 Porter St.
Frederick, MD 31702-5011
Phone: (301) 619 - 4947

Forwarding Address:

Dr. Leonard Wasieloski
6831 Acacia Court
Frederick, MD 21703

17) APPRAISAL OF THE ASSOCIATESHIP PROGRAMS

I completed my doctorate in 1995, and began my associatehip immediately after receiving my diploma. The associateship has been an outstanding foundation for me to begin my career. The research opportunities at USAMRIID have been excellent. I was able to work with leading scientist in my field, and direct my own project. The fellowship provided generous funding for travel and meetings. I had the opportunity to attend and present data at more, including international, meetings than I would have had in any other postdoctoral fellowship. I feel this has been invaluable in my career development.

Furthermore, the stipend and medical benefits have been excellent. And the staff of the associateships office has been extremely professional and helpful. I am very satisfied with my associateship experience.

9608630 AMRMC

DATE: 4/22/98

NAME: Sylva K. Yeghiayan, Ph.D.

NAME OR LABORATORY OR CENTER AND LOCATION:

Nutrition and Biochemistry Division
US Army Research Institute of Environmental Medicine
Kansas Street
Natick, MA 01760-5007

NAME OF RESEARCH ADVISOR: Harris R. Lieberman, Ph.D.

ARE YOU ON LEAVE FROM A PROFESSIONAL POST:

No

PROFESSIONAL SOCIETY OFFICES HELD DURING TENURE:

None

PROFESSIONAL TRAVEL DURING TENURE:

None

SEMINARS OR LECTURES DELIVERED AT UNIVERSITIES AND/OR INSTITUTES:

None

SUMMARY OF RESEARCH DURING TENURE:

I have completed a study on the effects of environmental heat stress on brain chemistry and behavior. Two dietary amino acids, tyrosine and arginine, were included in this study to determine the effects of these nutrients on stress-related neurochemical changes and behavioral parameters. Significant beneficial effects of tyrosine on heat stress-related performance were found.

During my tenure, I also wrote and received approval on a new protocol to study the effects of heat stress and altered macronutrient levels on neurochemistry as well as performance in a number of behavioral tasks. These studies, which are part of the Performance-Enhancing Ration Components (PERC) program, support STO V (Identification of Nutritional Strategies to Maintain Health and Soldiers' Performance) and STO B (Nutritional and Metabolic Requirements in Continuous Operations) objectives. Studies of this type contribute to ration design efforts intended to develop nutritional measures to counteract negative effects of intense stress, including harsh environmental conditions, associated with military operations and improve performance.

RESEARCH IN PROGRESS:

The first study on the new protocol described above is underway. Single-meal manipulations, as well as an eleven day diet treatment, will be conducted to assess the effects of altered macronutrient levels on such behavioral measures as the Porsolt Behavioral Despair Test, the Elevated Plus Maze and the Vogel Lick-Suppression Test. Additionally, neurochemical analyses will be conducted, as the dietary interventions may alter brain levels of tryptophan and serotonin.

PUBLICATIONS AND PAPERS RESULTING FROM NRC ASSOCIATESHIP RESEARCH:

Yeghiayan SK, Amendola C, Wu TH, Maher TJ and Lieberman HR. Hyperthermia and tyrosine administration affect behavioral performance but not hippocampal catecholamines, *Soc Neurosci Abstr* (submitted).

PRESENTATIONS AT SCIENTIFIC MEETINGS OR CONFERENCES

Poster to be presented at upcoming Society for Neuroscience meeting (November, 1998).

PATENT OR COPYRIGHT APPLICATIONS RESULTING FROM NRC ASSOCIATESHIP RESEARCH:

None

FUTURE POSITION AND ADDRESS AND/OR FORWARDING ADDRESS:

Please mail tax statement to:
Dr. Sylva Yeghiayan
122 Carroll Avenue
Westwood, MA 02090

APPRAISAL OR THE ASSOCIATESHIP PROGRAMS:

I enjoyed my tenure as an NRC Research Associate. The program provided for the freedom to pursue a line of research that is of interest to me and that can be built upon for some time to come. The program also appeared to be well-organized and I found the administrators to be easily approachable and accessible for questions. I believe that the Progress Reports are a good idea and, thankfully, there was a minimum of paperwork involved. I have only two comments on areas that could be improved. First, as part of a training experience, I believe it is useful to go to other laboratories to learn new techniques at times. I think it would be helpful if the program provided some flexibility for the location of the training. Also, as I mentioned in my resignation letter, I am bothered by the fact that early cessation of a tenure is the only solution for those needing a reduced work week. Again, more flexibility would be ideal. I believe that you would still find able and dedicated researchers on a part-time schedule and that there would be a mutual benefit for both these scientists and the NRC Research Associateship Program.